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Olfaction in the absence of adult brain serotonin

Kaitlin S. Carlson[*], Meredith S. Whitney[*], Marie A. Gadziola, Evan S. Deneris[#] and Daniel W. Wesson[#]

Department of Neurosciences, Case Western Reserve University, 2109 Adelbert Rd., Cleveland, OH, 44106, USA

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Co-first authors.

#Co-senior authors.

Correspondence should be addressed to either Daniel W. Wesson, dww53@case.edu or Evan S. Deneris, esd@case.edu

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Kaitlin S. Carlson[1,*], Meredith S. Whitney[1,*], Marie A. Gadziola[1], Evan S. Deneris[1,#], Daniel W. Wesson[1,#]

[1]Department of Neurosciences
Case Western Reserve University
2109 Adelbert Rd.
Cleveland, OH, 44106. U.S.A.

email for correspondence:
dww53@case.edu (D.W.W.) and esd@case.edu (E.S.D.)

*Co-first authors. #Co-senior authors.

Author contributions: KSC, MSW, ESD and DWW designed research. KSC and MSW performed research. KSC, MSW, MAG, ESD and DWW analyzed data and wrote the paper.

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Abstract

The neurotransmitter serotonin (5-HT) is considered a powerful modulator of sensory system organization and function in a wide range of animals. The olfactory system is innervated by midbrain 5-HT neurons into both its primary and secondary odor processing stages. Facilitated by this circuitry, 5-HT and its receptors modulate olfactory system function, including odor information input to the olfactory bulb. It is unknown, however, whether or not the olfactory system *requires* 5-HT for even its most basic behavioral functions. To address this question, we established a conditional genetic approach to specifically target adult brain *tryptophan hydroxylase 2 (Tph2*), encoding the rate-limiting enzyme in brain 5-HT synthesis, and nearly eliminate 5-HT from the mouse forebrain. Using this novel model, we investigated the behavior of 5-HT-depleted mice during performance in an olfactory go/no-go task. Surprisingly, the near elimination of 5-HT from the forebrain, including the olfactory bulbs, had no detectable effect on the ability of mice to perform the odor-based task. *Tph2* targeted mice were not only able to learn the task, but also had similar levels of odor acuity as compared to control mice when performing a coarse odor discrimination. Both groups of mice spent similar amounts of time sampling odors during decision-making. Furthermore, odor reversal learning was identical between 5-HT-depleted and control mice. These results suggest that 5-HT neurotransmission is not necessary for the most essential aspects of olfaction, including odor learning, discrimination, and certain forms of cognitive flexibility.

Significance statement

Modulation of sensory systems by neurotransmitters is considered critical for perception. The olfactory system is robustly innervated by 5-HT neurons into both its primary and secondary odor processing stages. Facilitated by this circuitry, 5-HT and its receptors modulate olfactory system function, including odor information input to the olfactory bulb. Here we asked whether the olfactory system needs 5-HT by using a conditional genetic approach to specifically target adult brain 5-HT synthesis and nearly eliminate 5-HT from the mouse forebrain. Our results suggest that 5-HT neurotransmission is not required for the most essential aspects of olfaction, including odor learning, odor discrimination, and odor-based cognitive flexibility. These findings raise questions about the importance and precise role of 5-HT modulation in olfactory system circuitry.

Introduction

Our sensory systems must encode information under a wide range of dynamic contexts for survival. One way the brain handles this task is to modulate the activity within local sensory processing centers by means of extrinsic substances. In this manner, top-down centers that produce neuromodulators can differentially release these modulators in sensory centers depending upon the needs of the animal. Major questions remain, however, regarding what neuromodulators are necessary for normal sensory system function (for reviews see (Katz, 1999; Hurley et al., 2004; Bouret and Sara, 2005; Linster and Fontanini, 2014)).

The neurotransmitter serotonin (5-HT) is considered a powerful modulator of sensory system organization and function in a wide range of animals (Hen, 1992; Jacobs and Azmitia, 1992; Cases et al., 1996). The mammalian olfactory system is innervated by 5-HT neurons into both its primary and secondary odor processing stages (McLean and Shipley, 1987; Smith et al., 1993; Steinfeld et al., 2015; Suzuki et al., 2015). Midbrain 5-HT neurons located in the dorsal raphe nucleus (DRN) and median raphe nucleus (MRN) innervate the olfactory bulb where fibers are observed in several cell layers (McLean and Shipley, 1987; Steinfeld et al., 2015; Suzuki et al., 2015; Muzerelle et al., 2016), including surrounding the glomeruli which represent the first synaptic processing stage of odor information.

Facilitated by this circuitry, 5-HT and its receptors modulate olfactory system function (McLean et al., 1993; Aungst and Shipley, 2005; Hardy et al., 2005; Petzold et

al., 2009; Liu et al., 2011a; Schmidt and Strowbridge, 2014; Brill et al., 2016; Brunert et al., 2016; Kapoor et al., 2016; Lottem et al., 2016)(For review see (Linster and Cleland, 2016)). For instance, electrical stimulation of the raphe nuclei modulates the level of odor information input into the olfactory bulb (Petzold et al., 2009). Additionally, optogenetic stimulation of raphe nuclei 5-HT neurons alters the representation of odors in several major populations of olfactory bulb neurons, including the primary output neurons (Brunert et al., 2016). Importantly, most of the above mentioned studies performed manipulations aimed at enhancing levels of synaptic 5-HT in the olfactory system. It is unknown, however, whether or not the olfactory system *requires* 5-HT for even its most basic behavioral functions.

In the present study, we investigated the behavior of mice with conditional depletions of adult brain 5-HT synthesis during their performance in an olfactory go/no-go task (Bodyak and Slotnick, 1999; Slotnick and Restrepo, 2001). To accomplish this, we used a recently established conditional approach (Whitney et al., 2016) to specifically target adult brain 5-HT synthesis, which nearly eliminates 5-HT from the entire mouse forebrain. Conditional targeting of adult brain 5-HT synthesis together with well-established operant methods to robustly assay olfactory psychophysics allowed to us test whether the adult olfactory system requires 5-HT for fundamental aspects of odor-guided operant behaviors, including odor learning and coarse odor discrimination. Our results suggest that adult brain 5-HT is not necessary for elementary function of the mammalian olfactory system.

Materials and Methods

Group design

Three different cohorts of *Tph2*$^{fl/fl}$ male mice (Kim et al., 2014) were utilized throughout this study (**Figure 1**). These cohorts provided opportunities to confirm the depletion of adult 5-HT using immunohistochemical, molecular, and chemical methods as well as to test the functional effects of 5-HT depletion using behavioral methods.

Surgical procedures and animal care

All animal procedures were in accordance with the guidelines of the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee at Case Western Reserve University. Young adult mice (~8 weeks of age) underwent a single survival intra-cranial surgical procedure to receive AAV as described in (Whitney et al., 2016). Following induction in Isoflurane anesthetic (3.0-3.5% in 1L/min O2), the mice were then mounted into a stereotaxic frame and anesthetic state maintained under Isoflurane. Core body temperature was maintained at 38°C with a heating pad. Upon confirmation of anesthesia depth, the head was shaved, cleaned with betadine and 70% EtOH, and a single injection of marcaine (S.C.) was administered within the site of the future wound margin. A single midline incision was made from ~3mm posterior of the nose along the midline to ~3mm posterior of lambda. Two holes (~1mm diameter) were drilled ±0.4mm from the midline of the skull (-4.15mm from bregma). A 10µL Gastight 1701 Hamilton syringe (30 gauge needle with a 13° bevel, Hamilton Company) loaded with AAV (either AAV1.CMV.PI.Cre.rBG or AAV1.CMV.PI.EGFP.WPRE.bGH, Penn Vector Core, Philadelphia, PA) was lowered into the intended injection site (-4.0mm ventral) and 1 µL of AAV infused at a rate of 100nL/min. Following the first injection, the syringe was slowly raised out of the brain and the process was repeated at the second site. After the second injection, the craniotomies and skull were closed. Rimadyl

158 (Carprofen, 5mg/kg, s.c., Pfizer animal health) was administered daily for 3 days post-
159 op. Food and water were available *ad libitum* except during behavioral recordings. All
160 animals were returned to group housing the day of surgery on a 12:12hr (light:dark)
161 schedule. Any mice exhibiting delayed recovery or signs of illness (lethargy, immobility,
162 ungroomed fur) throughout any point of experimentation were immediately euthanized
163 and not used for future data collection.
164
165 Histology
166 As illustrated in **Figure 1**, a cohort of mice (cohort 1) not used in the behavioral testing
167 was treated with AAV as described above and prepared for immunohistochemical
168 staining for 5-HT and Tph2. Two weeks post-surgery, the mice were anesthetized with
169 Avertin (0.5g tribromoethanol/39.5mL H_2O, 0.02mL/g body weight) and perfused with
170 cold phosphate buffered saline (PBS) for 2-5 minutes, followed by cold 4%
171 paraformaldehyde in PBS for 20 minutes. The brains were removed and cryoprotected
172 in 30% sucrose:PBS overnight. Next, frozen coronal sections through the olfactory bulb
173 and piriform cortex were obtained on a sliding microtome at 20µm thickness and were
174 placed in 0.3% sodium azide - Tris buffered saline at 4°C until staining. The remaining
175 midbrain and hindbrain tissue was left in 30% sucrose:formalin until sectioning until 20
176 µm frozen sections through all serotonergic nuclei were made on a sliding microtome.
177 Similar sections from AAV-GFP- and AAV-Cre-injected mice were mounted on slides
178 and vacuum-dried. They were then permeabilized in 0.3% Triton:PBS (PBS-T) and
179 blocked in 5% normal goat serum in PBS-T. Prior to blocking, antigen retrieval was
180 performed only on slides containing sections through the serotonergic nuclei due to

overfixation from formalin. Slides were placed in 10 mM sodium citrate, microwaved at low power for 10 minutes, cooled to room temperature, and washed 3x5 minutes in PBS. All slides were then incubated in primary antibody in blocking solution O/N at 4°C, washed 6x5 minutes in PBS-T, incubated in secondary antibody, and washed 6x5 minutes in PBS-T. Coverslips were mounted with ProLong® Gold antifade mountant with DAPI (Molecular Probes - Life Technologies). Primary antibodies used were rabbit anti-Tph2 (1:500, Millipore) and rabbit anti-5-HT (1:500, ImmunoStar), and the secondary antibody used was goat anti-rabbit Alexa 594 (Invitrogen). Coronal brain sections containing regions of interest were selected based upon established boundaries (Paxinos and Franklin, 2000). Sections were imaged on a Zeiss LSM510 confocal microscope or a Zeiss Axioskop II MotPlus. Digital inversion of the images in grayscale and brightness/contrast adjustments occurred in Adobe Photoshop equally for images within panels as noted (see **Figure 2**).

HPLC

Tissues from mice in cohorts 2 and 3 were prepared for HPLC analysis following established protocols (Lerch-Haner et al., 2008). Mice were anesthetized with Avertin and perfused with 10 U/mL heparin (Sigma Aldrich) in cold PBS for approximately six minutes to clear the brain of blood and remove confounding peripheral 5-HT. Brains were immediately removed and placed on dry ice. When they were partially frozen, the brains were cut at bregma -2.92 mm to separate the forebrain and then again directly posterior to the olfactory bulbs. The forebrain and olfactory bulbs were immediately

frozen on dry ice. Samples were shipped to the Neurochemistry Core of the Vanderbilt Brain Institute for processing and HPLC analysis of 5-HT and 5-HIAA levels.

qPCR

Mice from cohorts 2 and 3 were anesthetized with Avertin and perfused with 10 U/mL heparin (Sigma Aldrich) in cold PBS for approximately six minutes. Brains then were immediately removed and placed on dry ice. When they were partially frozen, the brains were cut at bregma -2.92 mm and -5.68 mm to isolate midbrain tissue, which contains the DRN. With the section in a petri dish on a cold plate, a 1.5mm tissue punch was made at midline, directly ventral to the third ventricle, to isolate the DRN. Tissue punches were lysed and homogenized using a 1mL dounce homogenizer, from which RNA was isolated using a PureLink® RNA Mini Kit (Ambion - Life Technologies). RNA was quantified using a NanoDrop 2000 (Thermo Scientific). 244 ng of RNA from each sample was used for reverse transcription to cDNA with a Transcriptor First Strand cDNA Synthesis Kit (Roche). *Tph2* and *Actb* levels were quantified by QPCR using TaqMan® Fast Advanced Master Mix with TaqMan® Gene Expression Assays for *Tph2* (Mm00557715_m1) and *Actb* (Mm00607939_s1) (Applied Biosystems – Life Technologies). The reactions were run in triplicate using a StepOnePlus™ system (Applied Biosystems) and relative expression values were calculated by StepOnePlus™ Software with *Tph2* levels normalized to *β-actin* expression.

Behavior

Mice used for olfactory go/no-go testing (cohorts 2 and 3, see **Figure 1**) were allowed three weeks to recover from surgery before any behavioral testing began. This duration also provided sufficient time for the AAV transduction and the AAV-Cre-mediated targeting of brain *Tph2* (see Results). Baseline bodyweights were then collected from all mice, and the mice were placed on a 24-hour water restriction schedule with water available every 24hrs in a small dish on their cage floor and/or in the context of behavioral task performance. We used a standard 80-85% body weight (from baseline weight) to ensure motivation in the operant water-motivated task (Slotnick and Restrepo, 2001). This level of weight loss is mild, and the mice appeared healthy (well-groomed fur, regular food intake) and active. After reaching 80-85% of baseline, mice were acclimated to the go/no-go operant boxes. Mice were single-housed for all behavioral procedures and all testing occurred during the light phase of the cycle (0900:1800 hours). All behavior was carried-out in a dimly-lit, well-ventilated room at 20-22°C.

We used three custom-built go/no-go operant chambers designed based upon the work of (Bodyak and Slotnick, 1999; Slotnick and Restrepo, 2001). The chambers were constructed out of ¼" thick ABS plastic (acrylonitrile butadiene styrene) and custom 3D printed (PLA, polyactic acid) nose-poke ports. The inner dimension of the chamber was 6x6" with 11" tall walls. On one wall was an operant plate consisting of two holes housing the two nose-poke ports (**Figure 3A**). The nose-poke ports were ¾" inner diameter and were positioned 2" apart (center to center) and each were 1.25" above the chamber floor (from floor to center of port). On another wall of the operant chamber was a hinged door allowing placement and removal of the mice. The

chambers were open-top with no ceiling to facilitate air circulation. The top of the odor port terminated into a flexible air hose (1" diameter) which was connected to a 12v computer fan, which drew air from within the chamber (through the poke poke) up through the fan. An infrared LED and infrared photodetector were placed ~2mm into each port to provide continuous measures of port entry (beam interruption). Finally, the chambers were each housed in a single wooden enclosure box each possessing an 8" wide 12v computer fan on one wall of the enclosure and a vent hole on the other to ensure air could freely circulate throughout the chamber and to facilitate odor elimination from within. All valves, computer hardware, and odor vials were housed outside of the enclosure boxes, which were positioned on a stainless steel rack.

Odor solenoid valves (Parker Hannifin, Cleveland, OH) and reward pinch valves (NResearch Inc., West Caldwell, NJ) were controlled by custom code written in Tucker-Davis Technologies software (Alachua, FL) which gated voltage through a relay driver module (LabJack Corp, Lakewood, CO). The status of the infrared nose-poke beams as well as valve activity (odor or reward) was relayed into a digital processor (Tucker-Davis Technologies, Alachua, FL) and then acquired to a computer at 3kHz sampling rate.

Mice were shaped in the go/no-go task across four phases (Bodyak and Slotnick, 1999; Slotnick and Restrepo, 2001). In phase 1, upon nose-poke into the odor port (in absence of odor), the mice were allowed to withdraw and then nose-poke into the reward port in exchange for the water reward (~3µl). For the first 2 blocks of this, to facilitate task acquisition, the nose poke into the odor port automatically triggered water reward release, without the need for the animal to even poke within the reward port. Throughout phase 1, the duration required for the mouse to hold its nose in the port

(break the beam) was gradually increased from 200 to 600ms in 200ms increments. Thus by the end of Phase 1 the mouse must be holding its nose in the odor port for 600ms. Upon achieving ≥85% correct responses in two consecutive blocks of 20 trials (criterion performance), the mice were switched into phase 2 of training. In phase 2, the mice were required to nose-poke for 600ms and then sustain the poke for an additional 200ms during which time a CS+ odor was delivered (800ms total hold requirement). The CS+ odor (see details on odors below) was delivered in all trials the animal held its nose in the port for ≥600ms. The mice were then gradually required to increase their hold duration to now remain with their noses in the port for 400ms of CS+ odor presentation (1000ms total hold requirement). Upon achieving criterion performance on phase 2, mice were transitioned to phase 3 wherein they had to detect the CS+ odor trials vs. blank stimulus trials. During both trials total hold duration was 1000ms (600ms, followed by 400ms stimulus). Withdrawal from the odor port and poking in the reward port during CS+ trials resulted in a water reward. Upon achieving criterion performance on phase 3, mice were transitioned to phase 4 and shaped on the odor discrimination task. In this, the mice were again required to nose poke in the odor port for a minimum of 1000ms (600ms, followed by 400ms stimulus). Withdrawal from the odor port and poking in the reward port during CS+ trials resulted in a water reward (hit). Delivery of CS+ but failure to poke in the reward port within 5 seconds was counted as an error (miss). Delivery of a CS- odor and failure to poke in the reward port within five seconds was counted as a correct reject. Finally, delivery of a CS-, followed by poking in the reward port within five seconds was counted as a false alarm. The % of correct responses (hits and correct rejects vs misses and false alarms) was determined for each block of 20 trials. During

phase 4, a minimum 5 sec inter-trial interval was enforced. Mice were not cued to nose poke but instead were able to self-initiate trials by nose poking beyond the boundaries of the inter-trial interval. The inter-trial interval could thus be reset following completion of new trials and/or due to 'short samples' wherein the animal failed to maintain nose poke for the required duration. All mice contributing complete phase 4 data in the study were required to complete the same number of blocks (100 ± 5) prior to going onto the reversal learning task. During the reversal task, the behavioral contingencies for the CS+ and CS- were switched so that the previous CS- is now a CS+ (rewarded) and *vice versa*.

Following reversal learning tests, the mice were required to perform in 6 blocks at or above criterion level (85%) prior to engagement in the odor discrimination self-regulation task wherein they were only mandated to nose poke for 600ms and continue to nose poke for 50ms of odor. In this overall testing structure, all mice needed to reach and demonstrate consistent criterion levels of behavior prior to being transitioned into the subsequent tasks.

Finally, after all olfactory testing, the mice were tested for water motivation on the subsequent day. For water motivation tests, mice were placed in the operant chambers for one hour wherein each nose poke into the odor port (in absence of odor) immediately triggered a reward to be released in the reward port on a fixed ratio 1 schedule.

The operant boxes were cleaned thoroughly with water and 90% EtOH between all behavioral sessions and mice and allowed to dry. Mice were only tested once per day.

317

318 Stimulus presentation

319 Odors were presented through a custom air-dilution olfactometer (see description
320 above) with independent PTFE stimulus lines up to the point of entry into the odor port.
321 Odorants included ethyl butyrate, heptanal, isopentyl acetate, (-)-limonene (Sigma
322 Aldrich, St. Louis, MO), each at their highest available purity (>97%) and each diluted to
323 0.5 Torr vapor pressure in mineral oil. These odors elicit distinct patterns of main
324 olfactory bulb activity (e.g., (Johnson et al., 2002), and thus they were selected for our
325 assay of 'coarse' olfactory discrimination. Odors and a 'blank' stimulus (mineral oil) were
326 presented at a rate of 1l/min. 2ml of liquid odor was aliquoted into 25ml glass
327 headspace vials sealed with Teflon septa (Shamrock Glass, Seaford, DE) and air flow
328 through the vials permitted with 18Ga s/s needles fit with PTFE (polytetrafluoroethylene)
329 lure fittings which terminated into PTFE odor lines (1/16" i.d.; Clippard Minimatic,
330 Cincinnati, OH). Not all animals were tested with all odors. One cohort of mice used for
331 behavior was initially shaped on one odor pair, whereas the other behavioral cohort was
332 shaped on a different odor pair. The experimenter was not blind to odor assignment, but
333 all stimulus presentation was automated. Rewarded and unrewarded odors were
334 pseudo-randomized within each block (10 trials of each CS+ and CS-). The
335 concentrations of odorants were selected to be well-above detection thresholds for mice
336 [21]. Odors were presented until the animal withdrew from the odor port or for a
337 maximum duration of 2000 ms.

338

339 Data analysis

Behavioral data acquired from the operant chambers were extracted in custom code written in Spike2 (Cambridge Electronic Design, Inc., Cambridge, England). Data were extracted by a single experimenter prior to the unblinding of this experimenter to treatment groups. All statistical tests were performed in StatView (SAS Institute, Inc., Cary, NC). Data were pooled across cohorts within measures, organized by treatment group (AAV-GFP vs AAV-Cre), and confirmed normally distributed with a Kolmogorov-Smirnov test. Statistical *p* values are 2-tailed unpaired *t*-tests unless otherwise specified. Values are reported as mean ± SEM unless otherwise indicated.

Results

Conditional targeting of adult brain 5-HT synthesis

Three cohorts of mice were used to validate adult brain-specific *Tph2* targeting and to test the necessity of adult brain 5-HT in odor-guided learning and olfactory perception (**Figure 1**). We utilized a recently established protocol (Whitney et al., 2016) to specifically target adult brain 5-HT synthesis by stereotaxic injection of an adeno-associated viral (AAV) Cre recombinase vector into mice (Kim et al., 2014) that have loxP sites flanking the fifth exon of *tryptophan hydroxylase 2* (*Tph2*$^{fl/fl}$), which encodes the rate-limiting enzyme for the production of 5-HT (**Figure 2A**). In adult *Tph2*$^{fl/fl}$ mice, AAV-Cre or AAV-GFP was injected into the midbrain where 5-HT neurons of the median and dorsal raphe nuclei (MRN/DRN) are located and project to olfactory structures in the forebrain (McLean and Shipley, 1987; Steinfeld et al., 2015).

In an independent cohort of *Tph2*$^{fl/fl}$ mice, we performed anti-5-HT and anti-Tph2 immunostaining to verify the targeting of *Tph2* and 5-HT depletion following injection of

either AAV-Cre or AAV-GFP into the mice at 6 weeks of age (cohort 1, *n* = 2 and 4, respectively) (**Figure 2**). Analyses performed two weeks following injection of AAV-Cre into *Tph2*$^{fl/fl}$ mice indicated a near-complete loss of 5-HT and Tph2 immunoreactivity in the MRN and DRN, as compared to AAV-GFP-injected mice (**Figure 2B**). In contrast, and as an example of the precision of this approach, 5-HT and Tph2 immunoreactivity were preserved in the medullary raphe, which provide 5-HT innervation to the spinal cord (Bowker et al., 1981; Skagerberg and Björklund, 1985) (**Figure 2C**). While virally-mediated Cre expression is not restricted to 5-HT neurons, this targeting is specific to the 5-HT system as *Tph2* is only expressed in 5-HT-producing neurons (Walther et al., 2003). Importantly, 5-HT-immunopositive fibers were strikingly absent in major olfactory structures, including in all the cell layers of main olfactory bulb and piriform cortex (**Figure 2D**). Particularly in the olfactory bulb, the massive amounts of anti-5-HT fibers originating in the MRN and terminating in the glomerular layer (McLean and Shipley, 1987; Steinfeld et al., 2015; Suzuki et al., 2015; Muzerelle et al., 2016), the first synaptic processing layer of odor information, were absent in AAV-Cre-injected *Tph2*$^{fl/fl}$ mice (**Figure 2D**). A rare and isolated 5-HT immunopositive fiber was observed in occasional AAV-Cre-treated *Tph2*$^{fl/fl}$ mouse brain sections, including in the olfactory bulb granule cell layer. While not shown here, 5-HT fibers were also absent in other olfactory structures, including the olfactory tubercle and anterior olfactory nucleus. Thus, as reported recently (Whitney et al., 2016), treatment of adult *Tph2*$^{fl/fl}$ mice with AAV-Cre achieves a near-complete loss of brain 5-HT, including, as shown here, in the olfactory bulb and piriform cortex.

Learning and performance of adult Tph2 targeted mice in an olfactory go/no-go task

Having verified a conditional approach for the near-complete elimination of 5-HT in the adult forebrain including olfactory structures, we next sought to investigate our main hypothesis that adult brain 5-HT is necessary for essential odor-guided behaviors. There are countless assays available to explore olfactory perceptual function in mice. We selected the well-established, nose-poke-based olfactory go/no-go task (Bodyak and Slotnick, 1999; Slotnick and Restrepo, 2001) based upon (Pfaffmann et al., 1958) (**Figure 3**). This operant task requires water-restricted mice to perform an instrumental water-motivated response to a conditioned odor and, throughout conditioning, the behavior of the animal is motivated by thirst versus odor-specific motivation. This is important since odor-specific motivation may alter odor investigatory dynamics (sniffing), a variable that may be compounded by 5-HT manipulations, which may modulate motivated behavior (Liu et al., 2014). In the go/no-go task, animals must learn to engage in the operant behavioral sequence of nose poking into an odor port to allow the possibility of receiving a 3µl drop of water from the neighboring reward port. Reward delivery in the reward port only occurs upon nose pokes into this port preceded by the conditioned rewarded odor (CS+), but not the conditioned unrewarded odor (CS-) (**Figure 3A**). CS+ and CS- odor trials occurred in a pseudo-random order throughout all testing sessions.

Training in the go/no-go task occurs over four phases (see Materials and Methods), and thus a potential initial hurdle in our investigation into the olfactory behavior of 5-HT depleted mice was shaping them to criterion performance (≥85% correct responses in 2 consecutive blocks of 20 trials). Indeed, 5-HT is considered a

potent modulator of odor learning in neonatal rats (McLean et al., 1993). As before, six-week-old adult *Tph2*$^{fl/fl}$ mice (cohorts 2 & 3) were injected with AAV-GFP or AAV-Cre into the midbrain DRN/MRN region. Mice were placed on a mild 24-hour water restriction schedule at 23-29 days post-treatment to allow sufficient time for the near-complete elimination of brain 5-HT levels before shaping on the task started (see timeline in **Figure 1**). One AAV-Cre-treated mouse was eliminated from all data analysis and statistical reports herein following both 5-HT HPLC and *Tph2* qPCR results that revealed intact brain 5-HT and *Tph2* levels, likely due to imprecise AAV injection.

Impressively, all remaining mice in the AAV-GFP (n = 9) and AAV-Cre (n = 8) treatment groups were able to learn the go/no-go task (**Figure 4**). *Tph2*-targeted mice required a similar number of training blocks as control mice to learn reward retrieval in Phase 1 (**Figure 4A**) ($t(15)=-0.655$, $p=0.522$), sampling of the CS+ odor in Phase 2 (**Figure 4B**) ($t(15)=-0.028$, $p=0.978$), and odor detection of the CS+ odor from a "blank" stimulus in Phase 3 (**Figure 4C**) ($t(15)=-0.724$, $p=0.480$). When learning to discriminate the CS+ odor from a CS- odor in Phase 4, *Tph2*-targeted mice required significantly more training blocks to reach performance criterion (**Figure 4D**) ($t(15)=3.507$, $p=0.003$). However, this finding was largely attributable to longer learning latencies in just two of the eight AAV-Cre mice which eventually exceeded the criterion threshold (**Figure 4D**, downward arrowheads).

With both groups of mice having learned the go/no-go task, we next allowed them to perform in the odor discrimination phase over multiple successive daily sessions (range: 5 – 9 sessions) for a total of 2,000 trials each (100 blocks / mouse). 100 blocks of performance was selected as a behavioral milestone to allow a large

433 sampling of behavior from all animals and to ensure extensive, as well as equivalent,
434 task experience prior to engaging in the upcoming reversal learning paradigm. One
435 AAV-Cre- and one AAV-GFP-treated mouse were eliminated from this and further data
436 acquisition due to complications occurring within the two weeks of behavioral shaping,
437 which impaired performance in the task (see **Figure 1**). As shown in **Figure 4E**, the
438 remaining AAV-Cre mice achieved similar behavioral performance as AAV-GFP mice
439 on the odor discrimination task, whether the mandatory odor sampling time was fixed at
440 400ms ($t(13)=-1.073$, $p=0.306$) or was self-regulated in a less restricted task structure
441 (see Materials and Methods) performed across 15 blocks on subsequent sessions
442 ($t(13)=-1.486$, $p=0.161$). Similarly, over the course of the first 15 blocks of criterion
443 performance, AAV-Cre mice sampled odors, measured as the time from odor onset to
444 withdrawal from the sampling port, for similar durations compared to AAV-GFP mice
445 (**Figure 4F**), in both the original odor discrimination task ($t(13)=0.577$, $p=0.574$) and
446 when odor sampling was self-regulated ($t(13)=1.358$, $p=0.198$). Thus, while both groups
447 sampled odors for qualitatively less time in the self-regulated task compared to the
448 original fixed odor sampling sessions (**Figure 4F**, right vs left), AAV-Cre mice
449 maintained similar odor discrimination accuracy and odor sampling times to AAV-GFP
450 mice on both task structures.

451 In a separate session, we sought to confirm that the mice were indeed relying
452 upon olfactory cues to perform the go/no-go task. In this experiment, a subset of mice (n
453 = 7) shaped to criterion performance on the CS+ vs. CS- Phase 4 odor discrimination
454 task were tested for their reliance upon the odors to make correct responses. Following
455 two blocks of CS+ vs. CS- odor discrimination (**Figure 4G**, shaded), the experimenter

disconnected the odor input lines to the odor ports of the operant chamber (**Figure 4G**, unshaded. The reduced performance upon block three and proceeding into block four falls within chance levels (<60% correct responses) in all mice. Upon block five, the experimenter reconnected the odor lines and the performance of the mice gradually returned (**Figure 4G**, shaded). This illustrates that the mice were indeed using the odors to guide their behaviors, not other cues that may be associated with the operant chamber function (valve clicks, air flow, etc).

Gross motor control, water intake, body weight, and water motivation of Tph2-targeted mice

In order to identify any additional factors which may influence go/no-go task performance in adult *Tph2*-targeted mice, we performed further measures on the mice (cohorts 2 and 3) during either task engagement or following completion of all olfactory testing. First, we measured gross motor control during task performance as assayed by the average duration of withdrawal from the odor port until nose poke in the reward port in phase 4 odor discrimination testing (**Figure 5Ai**) and during the self-regulation odor discrimination testing (**Figure 5Aii**). These data revealed that *Tph2*-targeted mice maintained coordinated performance (nose poking and movement between ports) in both phase 4 ($t(15)=1.809$, $p=0.198$) and the self-regulation testing ($t(13)=0.548$, $p=0.593$) as compared to the AAV-GFP treated mice.

We also monitored body weights and fluid intake of the water restricted mice. AAV-Cre- and AAV-GFP-treated mice began water restriction at statistically similar baseline body weights (**Figure 5B**) ($t(15)=-1.456$, $p=0.165$). Importantly, all mice were

maintained at statistically similar body weights throughout go/no-go task performance (**Figure 5C**) (F (t(15)=-0.043, p=0.966). Further, we measured the amount of water provided to the mice each day following behavioral testing each day in order to maintain appropriate body weights (as shown in **Figure 5C**). Each day, the experimenter provided 'supplemental' water to the water-restricted mice (that in addition to what they received upon making correct decisions). AAV-Cre-injected mice required more water than AAV-GFP-injected mice to maintain 80-85% body weight levels throughout the duration of experimentation (**Figure 5D**) (t(15)=3.913, p=0.0014). Pilot experiments with a cohort of mice (not included in **Figure 1**) in which we provided AAV-Cre-injected mice with the same levels of water as AAV-GFP-injected mice revealed excessive weight loss in AAV-Cre-injected mice (data not shown) and therefore we established the paradigm of providing AAV-Cre treated mice with additional supplemental water each day in order to ensure mice in both treatment groups fell within a similar operational definition for thirst.

Finally, on a separate day (see **Figure 1**), mice were placed in the operant chambers for a test of water motivation lasting one hour. In this task, each nose poke into the odor port (in absence of odor) immediately triggered a reward to be released in the reward port, and, thus, this task allowed free access to water and quantification of water motivation. AAV-Cre-treated mice completed trial blocks with similar latencies (**Figure 5Ei**) (t(13)=-1.762, p=0.102) and completed a similar number of blocks (**Figure 5Eii**) (t(13)=1.76, p=0.102) compared to AAV-GFP-treated mice. Together, these data from the above control measures suggests that 'non-olfactory' behaviors of *Tph2*-targeted mice during engagement in the go/no-go test are not confounded by

502 differences in the ability of these mice to execute coordinated motor behaviors nor by
503 altered levels of water motivation.
504
505 *Preserved higher-order odor-guided behaviors in Tph2-targeted mice*
506 We next sought to explore the ability of *Tph2*-targeted mice to engage in 'higher-
507 order' olfactory-based behaviors. Reversal learning is a cognitive ability that often is
508 explored in the context of learning and memory studies. Being reliant upon the basic
509 function of odor detection and identification, odor reversal learning, wherein the
510 behavioral contingencies between the CS+ and CS- odors are switched so that the
511 previously unrewarded odor is rewarded and *vice versa*, is a commonly employed
512 paradigm (Macrides et al., 1982; Schoenbaum et al., 1999; Sokolic and McGregor,
513 2007). 5-HT is considered in some contexts to be essential for odor learning (McLean et
514 al., 1993) and cognitive flexibility (Lapiz-Bluhm et al., 2009; Coccaro et al., 2011). Thus,
515 while not specifically assaying olfactory perception, we next investigated reversal
516 learning to explore if this function of olfactory sensory-dependent cognitive flexibility is
517 impaired in 5-HT-depleted mice.
518 We reversed the CS+ and CS- behavioral contingencies and monitored the
519 responses of both AAV-GFP (n = 8) and AAV-Cre mice (n = 7) over successive daily
520 sessions (**Figure 6A**, all mice from cohorts 2 and 3). As was the case for the initial
521 learning of the go/no-go task (**Figures 4A-D**), both groups of mice displayed similar
522 learning curves to acquire the reversal (**Figure 6A**), with inter-animal variability present
523 in both groups. To reduce bin noise, learning curves were plotted and analyzed from
524 sliding block window averages over 3 successive blocks. AAV-GFP- and AAV-Cre-

525 injected mice required a similar number of sliding blocks to acquire the reversal to
526 criterion level (**Figure 6B**) (t(13)=1.067, p=0.305). Thus, at least in this context, mice
527 maintain cognitive flexibility in the absence of adult brain 5-HT.
528
529 *Confirmation of 5-HT synthesis deficiency in mice with perseverant olfactory behaviors*
530 *and evidence that residual brain 5-HT does not contribute to go/no-go behavior.*
531 We next verified loss of *Tph2* expression in the DRN of AAV-Cre injected *Tph2*$^{fl/fl}$
532 mice used for the go/no-go testing (cohorts 2 and 3, n = 9 AAV-GFP and n = 8 AAV-
533 Cre). Analysis of these mice demonstrated that this targeting approach resulted in a
534 98.01% decrease in *Tph2* mRNA in the DRN (t(15)=11.254, p<0.0001); AAV-Cre vs.
535 AAV-GFP mice) (**Figure 7A**). Further, the brain of each mouse was collected to confirm
536 the depletion of 5-HT (n = 9 AAV-GFP and n = 8 AAV-Cre). HPLC analysis confirmed
537 robust depletions of 5-HT and in its main metabolite, 5-hydroxyindoleacetic acid (5-
538 HIAA) in both olfactory bulb (5-HT, (t(15)=12.588, p<0.0001); 5-HIAA, (t(15)=12.519,
539 p<0.0001)) and forebrain (5-HT, (t(15)=19.847, p<0.0001); 5-HIAA, (t(15)=6.043,
540 p<0.0001)) (**Figures 7B & C**).
541 As we have presented (**Figures 2 & 7**), this approach yielded near-complete
542 eliminations of forebrain and olfactory bulb 5-HT. While highly significant reductions in
543 5-HT were present in AAV-Cre-injected mice compared to AAV GFP mice across all
544 animals in the olfactory bulb and forebrain, respectively, some 5-HT remained. Does the
545 residual 5-HT, that is, the minor amount found in the olfactory bulbs or forebrain
546 following AAV-Cre injection, contribute to odor discrimination as assayed here? To test
547 this, we compared, within AAV-Cre-injected animals, the HPLC-determined

concentration of residual 5-HT in both olfactory bulb and forebrain homogenates (same data as in **Figure 7B**) to that of each animal's percent correct responses (computed across 2,000 trials [same data as in **Figure 4E**]). Again, supporting the notion that the olfactory system does not need 5-HT for its basic function, no relationship was observed between residual 5-HT in either the olfactory bulb (Pearson's r = 0.321, $p = 0.483$, $n = 7$) or forebrain (Pearson's r = 0.0964, $p = 0.837$, $n = 7$) and the percent of correct responses.

Discussion

In the present study, we sought to address the longstanding question: Do mammals *need* 5-HT for olfaction? More specifically, without adult brain 5-HT, do animals possess deficits in the most basic elements of olfactory-guided behaviors? The olfactory system is innervated by numerous neuromodulatory systems. These systems, including dopamine, acetylcholine, and, as explored herein, 5-HT, are hypothesized to provide critical refinements to the function of the olfactory system within the olfactory bulb, as well as in secondary and tertiary olfactory processing stages (for review see (Linster and Fontanini, 2014)). For instance, multiple lines of elegant work have revealed that cholinergic modulation within the olfactory bulb and downstream piriform cortex impacts fine odor discrimination, short-term odor memory, odor-based rule learning, and odor habituation (e.g., (Ravel et al., 1992; Saar et al., 2001; Fletcher and Wilson, 2002; Linster and Cleland, 2002; Mandairon et al., 2006; Chaudhury et al., 2009)). Despite intense interest in understanding serotonergic modulation of olfactory

sensory input, surprisingly few studies have addressed the necessity of 5-HT in olfactory-guided behaviors in the intact animal. In one study, the serotonergic neurotoxin 5, 7-dihydroxytrypmaine (5,7-DHT) was used to destroy 5-HT axonal inputs to the rat olfactory bulb (Moriizumi et al., 1994). The authors reported that 5,7-DHT treated rats, trained to avoid cycloheximide in drinking water, became 'anosmic' after neurotoxin treatment (Moriizumi et al., 1994). However, the behavioral assay was not specific for olfaction and the reported behavioral impairment did not occur until several weeks after depletion of 5-HT immunoreactivity in the bulb. Moreover, the timing of anosmia was correlated with glomerular atrophy that also occurred a few weeks after 5,7-DHT treatment suggesting that the behavioral deficits were not the result of 5-HT deficiency but rather long term toxic effects of 5,7-DHT on olfactory structures. A different group reported that 5,7-DHT based denervation of centrifugal raphe input to the rat neonatal olfactory bulb is required for odor learning and memory, concluding a functional role of 5-HT in this context (McLean et al., 1993). However, in *Lmx1b* deficient mice, in which 5-HT neurons fail to differentiate and 5-HT synthesis is genetically blocked permanently at embryonic day 10.5, olfactory function remains intact (Liu et al., 2011b). The selective serotonin-reuptake inhibitor, fluoxetine, rescued deficits in olfactory acuity brought about in mice chronically treated with corticosterone (CORT) but impaired olfaction when chronically administered to CORT-free mice (Siopi et al., 2016). Thus, the significance of 5-HT, *itself*, specifically in the adult brain for odor-guided behaviors and also olfactory perception is unclear. This void in part comes from a lack of methods to specifically target adult brain 5-HT synthesis.

In this study, we used a sensitive olfactory go/no-go test and conditionally-targeted Tph2 with AAV-Cre injections in the adult brain to specifically block 5-HT synthesis thus avoiding potential off-target effects of pharmacological approaches. The olfactory go/no-go test is a sensitive assay of olfactory function, and qualitatively mice in our task learned and performed within ranges reported by other groups (Bodyak and Slotnick, 1999; Kelliher et al., 2003; Abraham et al., 2004; Lin et al., 2004; Pho et al., 2005; Wesson et al., 2006). Although the targeting of Tph2 caused a nearly complete elimination of 5-HT synthesis, this deficiency does not result in loss of 5-HT neuron cell bodies as evidenced by their intact expression of aromatic amino acid decarboxylase (Whitney et al., 2016). The conditional genetic approach left intact early 5-HT signaling and thus did not interfere with 5-HT's effects on sensory system organization and development (Cases et al., 1996; Gaspar et al., 2003; Toda et al., 2013). Further, this approach avoids potential but presently unknown compensatory mechanisms (Whitney et al., 2016) that might occur in response to 5-HT deficiency in the developing nervous system. The blockade of adult 5-HT synthesis did result in a significant and robust phenotype of altered water motivation (**Figure 5**), and has been shown to impact activity levels and circadian rhythms (Whitney et al., 2016). However, in view of the profound effects of serotonergic function on olfactory circuitry the present results were unexpected as they indicate that adult brain 5-HT is not necessary for odor learning, coarse odor discrimination, or normal coordinated odor-guided behavior (sampling durations, **Figure 4F**). Further, we found that Tph2-targeted mice were able to successfully complete a reversal learning task (**Figure 6**). This finding was also surprising as a significant number of studies employing a range of approaches to alter

5-HT function and then assess behavioral outcomes in rodents, non-human primates, and humans, have repeatedly suggested that reduced brain 5-HT levels hinders reversal learning (Murphy et al., 2002; Masaki et al., 2006; Bari and Robbins, 2013; Izquierdo et al., 2016). A potential explanation for our discordant findings is that the rat, not the mouse, has been the typical rodent species used to probe 5-HT's role in reversal learning. In addition, because perturbation of different 5-HT receptor subtypes, which presumably influence distinct circuitry, produces opposing effects on reversal learning (Furr et al., 2012; Nilsson et al., 2012), perhaps a near-complete absence of forebrain 5-HT has a net counterbalancing effect on reversal learning. A similar explanation may account for the lack of effect of adult brain Tph2 targeting on olfaction. Yet, with respect to reversal learning outcomes this explanation does not account for the discrepancy between our findings and earlier ones in which 5-HT was also broadly depleted from the forebrain and therefore would have been expected to impact 5-HT signaling through more than one 5-HT receptor subtype. A conspicuous difference between our study and earlier ones, however, is the method used to deplete brain 5-HT. In contrast to our viral/genetic approach, previous studies have employed pharmacological or dietary means to reduce brain 5-HT (Clarke et al., 2004, 2005, 2007; Masaki et al., 2006; Lapiz-Bluhm et al., 2009; Bari et al., 2010; Izquierdo et al., 2012, 2016). Although off-target pharmacological effects are not potentially inherent in our Tph2 targeting approach we cannot rule out the possibility that the severe deficiency of brain 5-HT in Tph2 conditional knockout mice lasting several weeks before behavioral testing may have triggered presently unknown secondary effects, such as homeostatic alterations in the function of other transmitter systems, that compensate for the absence

639 of 5-HT modulation of olfactory synaptic circuitry. Further parallel investigations of
640 cognitive flexibility with adult *Tph2*-targeted mice and traditional methods of 5-HT
641 depletion are needed to resolve the mechanistic basis of previously reported effects,
642 which our results suggest, are not directly due to altered 5-HT levels.

643 Our findings beg the question as to what role 5-HT plays in olfaction? Why do the
644 essential olfactory structures receive massive 5-HT innervation if not for even adjusting
645 the most essential functioning of the system? One possibility is that 5-HT is only needed
646 for the more nuanced aspects of odor-guided behavior, including the discrimination of
647 structurally similar odorants (Cleland et al., 2002; Uchida and Mainen, 2003; Abraham
648 et al., 2004), figure-background segregation (Barnes et al., 2008; Chapuis and Wilson,
649 2011), or intensity perception (Wojcik and Sirotin, 2014). For instance, a mouse may
650 need 5-HT to discern the difference of odor in a sexually receptive female mouse versus
651 an unreceptive one, yet may not need 5-HT to recognize the scent difference between a
652 mouse and a predator. It is also possible that the influence of 5-HT would only manifest
653 in cases of changes in behavioral state, including startle, arousal, or hunger.
654 Nevertheless, our results that the essential functions of the mammalian olfactory system
655 can persevere despite a near-complete absence of adult brain 5-HT are striking and
656 suggest need for a refinement of models wherein 5-HT serves a major role in the
657 olfactory system. Instead, we may benefit from recognizing a far-more limited role of 5-
658 HT in olfactory system function and perception. Further, this work provides a foundation
659 to probe for compensatory synaptic mechanisms whereby our olfactory system may
660 engage in informing behaviors, including those involving transmitter co-release
661 (Trudeau, 2004; Zhou et al., 2005; Barker et al., 2016).

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Figure Legends

Figure 1. Experimental timeline for all mouse cohorts. Three separate cohorts of *Tph2*$^{fl/fl}$ male mice were used. **(A)** One cohort (cohort 1) was used solely for immunohistochemistry. Two other cohorts (cohorts 2 and 3) **(B)** were used for olfactory go/no-go behavioral experiments, and following the behavioral data collection, perfused for subsequent qPCR and HPLC analyses of *post-mortem* brain tissue (see Materials and Methods). Two mice from the behavioral group (cohorts 2 and 3) were euthanized/died during the late stages of task acquisition upon displaying signs of illness (indicated by stars).

Figure 2. Targeting of *Tph2* in the brains of adult mice. (A) Schematic of wild-type *Tph2* allele (top), floxed-allele with exon V flanked by *loxP* sites (middle), and targeted allele after Cre-mediated deletion of exon V (bottom). **(B)** Representative images of $Tph2^{fl/fl}$ mice confirming near-complete loss of 5-HT and Tph2 immunoreactivity within the MRN and DRN (median and dorsal raphe nucleus) of AAV-Cre-treated mice. **(C)** Representative images of $Tph2^{fl/fl}$ mice illustrating the preservation of 5-HT- and Tph2-immunostained neurons in the medullary raphe, including the raphe magnus (RMg) and raphe pallidus (RPa) in AAV-Cre-treated mice. The raphe magnus does not send many fibers into the forebrain and/or olfactory structures specifically, but instead largely projects into the spinal cord (Bowker et al., 1981; Skagerberg and Björklund, 1985). **(D)** Representative images of $Tph2^{fl/fl}$ mice confirming near-complete loss of 5-HT immunoreactivity within both the main olfactory bulb and piriform cortex in AAV-Cre-treated mice. Images are from stacks of 1.2µm thick confocal images. Dashed lines in olfactory bulb images represent borders of glomeruli identified with nuclear counterstain (not shown) and in piriform lines represent the border of the lateral olfactory track (lot) form layer i of the piriform. Images are from coronal sections, 20µm thick. Images have been converted to monochrome and inverted. Images in (B) and (C) were equally applied contrast/brightness adjustments to optimally display immunostained cell bodies. Images in (D) were similarly equally applied contrast/brightness adjustments to optimally display immunostained fibers.

Figure 3. Go/no-go olfactory task design and stimulus control. (A) Outline of the go/no-go task structure as described in detail in Materials and Methods. In the final stage of the task, mice nose-poke in the right port (600ms hold duration required), receive either CS+ or CS- (400msec minimum hold [50msec for self-regulation paradigm]; 2sec maximum hold), and retrieve a reward in the left port (S+ trial) or withhold their response (S- trial). Green shaded circle denotes the presentation of a conditioned rewarded (CS+) odor. Red shaded circle denotes the presentation of a conditioned unrewarded (CS-) odor. Blue water-drop icon symbolizes brief 3µL water reward delivery. **(B)** Averaged voltage trace from a photoionization detector (PID) to illustrate the rapid odor stimulus dynamics as controlled by the go/no-go olfactometers.

15 trials of the odorant, heptanal (see Materials and Methods for intensity used and flow rate), were delivered by each olfactometer while the PID sampling port was positioned in the center of the odor sampling port. Data are normalized to the maximum value acquired by each olfactometers averaged PID output (over the 15 trials) and plotted as the average across all three olfactometers. Time 0 equals odorant valve onset. Time points of 50% (T_{50}) and 90% rise times (T_{90}) are indicated. Grey-shaded area indicates SEM. While these dynamics may vary slightly across odors, this measure illustrates the precision and stability of the odor presentation methods employed.

Figure 4. Adult Tph2 targeted mice learn the olfactory go/no-go task and display similar levels of odor acuity and odor sampling durations. (A-D) Learning curves (left) and total number of blocks required to complete each phase (right) across Phases 1-4. $^{**}p<0.005$. **(E)** Average block percent correct performance during the odor discrimination task when odor sampling time was fixed (left, 2000 trials/mouse) and self-regulated (right, 300 trials/mouse). **(F)** Average sampling durations (nose poking during odor on) for CS+ and CS- odors during Phase 4 odor discrimination (blocks ≥85%, 300 trials/mouse (left), and during self-regulation of odor discrimination (blocks ≥ 85%, 300 trials/ mouse) (right). **(G)** Odor-removal control experiment to demonstrate the reliance of the mice upon the odor stimuli to engage in the go/no-go task. Data = mean +/- SEM of all mice / block. Dots = individual mouse data.

Figure 5. Gross motor performance, body weights, water intake, and water motivation of AAV-GFP- and AAV-Cre-treated *Tph2*$^{fl/fl}$ mice during the go/no-go task (A) Average duration of withdrawal from odor port to nose poke in the reward port during **(Ai)** Phase 4 odor discrimination (15 blocks ≥ 85%, 150 CS+ trials/mouse) and **(Aii)** the self-regulation odor discrimination testing (15 blocks ≥85%, 150 CS+ trials/mouse). **(B)** Baseline body weights of mice used in olfactory go/no-go testing prior to water deprivation. **(C)** Mean body weights of all mice (averaged across all days of behavioral testing (range: 21-24 days) expressed as % of weight (during water restriction) as a function of their baseline weight (A). **(D)** The mean of supplemental water for each mouse across all testing days. **(E)** Water motivation test results. **(Ei)**

Histograms of the average block duration (11-30 blocks/mouse) and **(Eii)** the number of blocks completed in a single one-hour session of the water motivation test. Data = mean +/- SEM of all mice. Dots = individual mouse data. $^{**}p<0.005$.

Figure 6. Adult brain 5-HT synthesis is not required for olfactory reversal learning. (A) Learning curves during odor-pair reversal, plotted with a three-block average sliding window until each mouse reached or surpassed 85% correct responses. **(B)** Average number of sliding blocks to reach ≥ 85% correct. Circles indicate values for individual mice.

Figure 7. Confirmation of Tph2 targeting and 5-HT depletion in cohorts used for behavior experiments. (A) qPCR results displaying a significant reduction in *Tph2* expression, relative to *Actb*, in the DRN region of $Tph2^{fl/fl}$ mice injected with AAV-Cre (n = 8) as compared to untreated controls (n = 9). $^{***}p<0.0001$. Data are from mice in cohorts 2 and 3. HPLC-quantified levels of 5-HT **(B)** and 5-HIAA **(C)** in the olfactory bulbs and forebrain of the same mice used for go/no-go behavior (cohorts 2 and 3). Tissue were collected immediately following the completion of the last behavioral measure (water motivation, Figure 5E). Data = mean ± SEM, individual points = individual mice. $^{***}p<0.0001$.

Figure 1

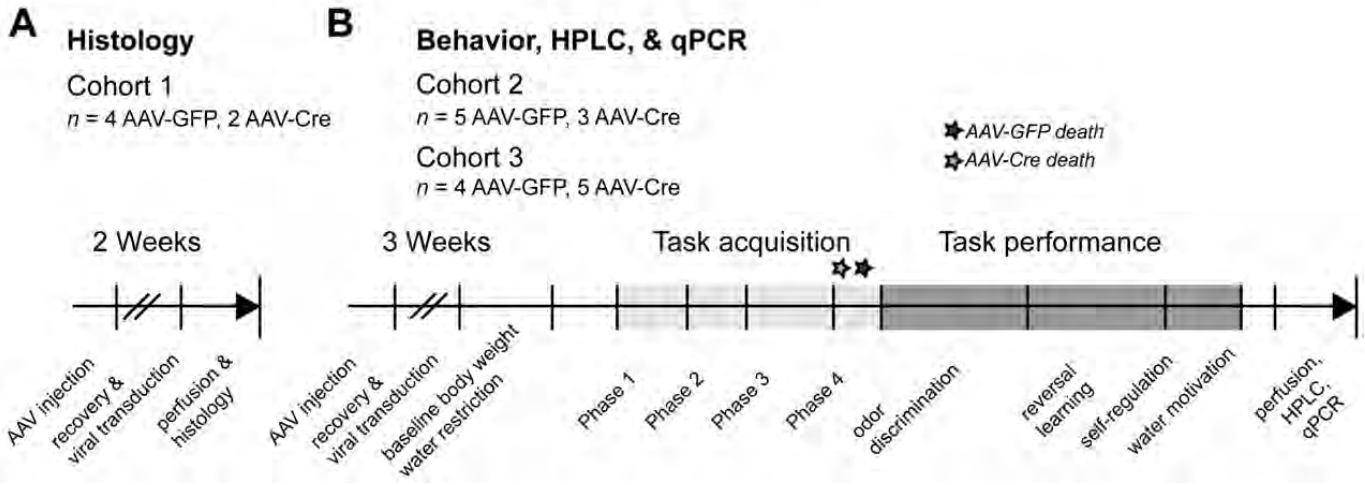

Figure 2

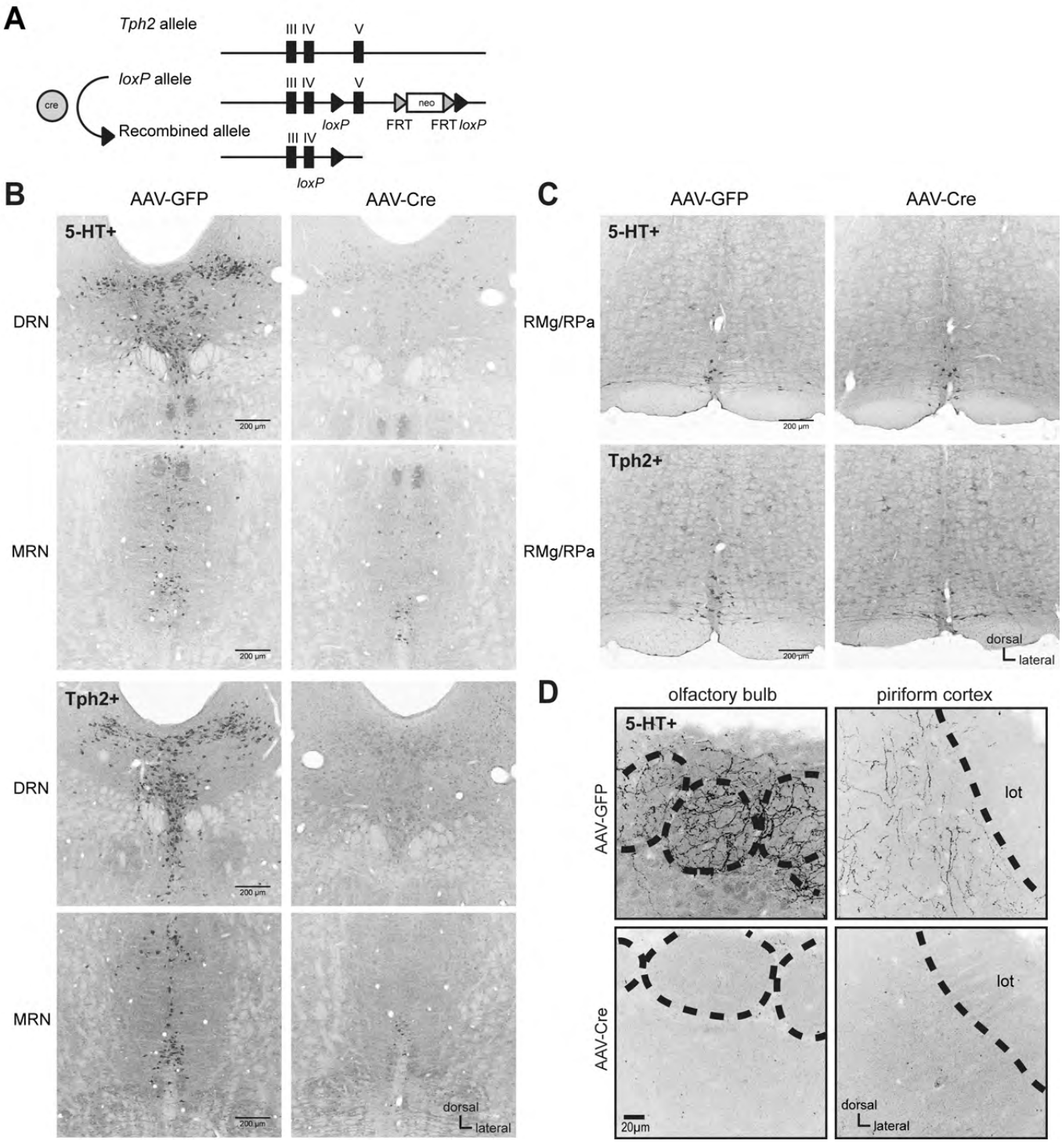

Figure 3

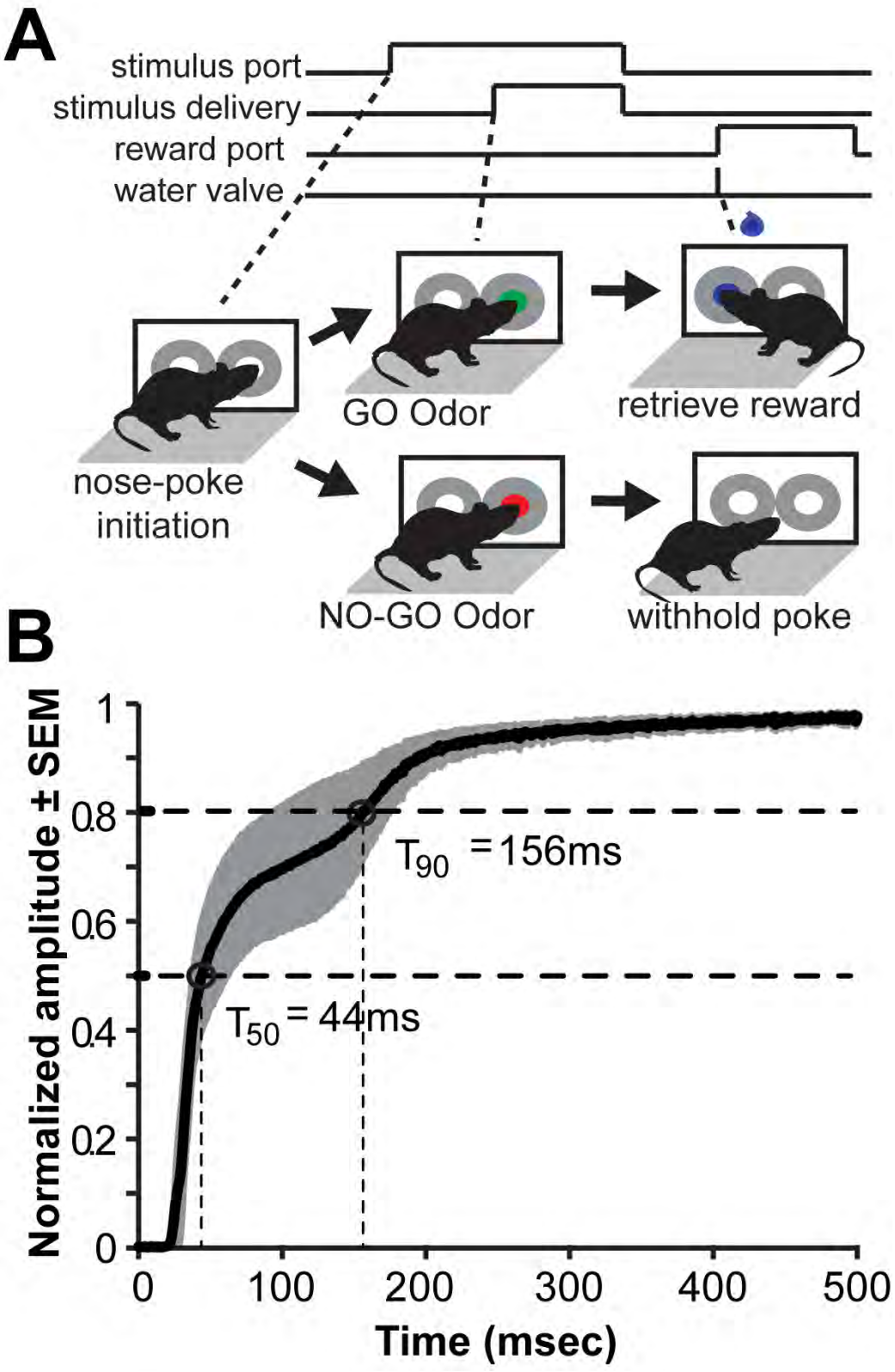

Figure 4

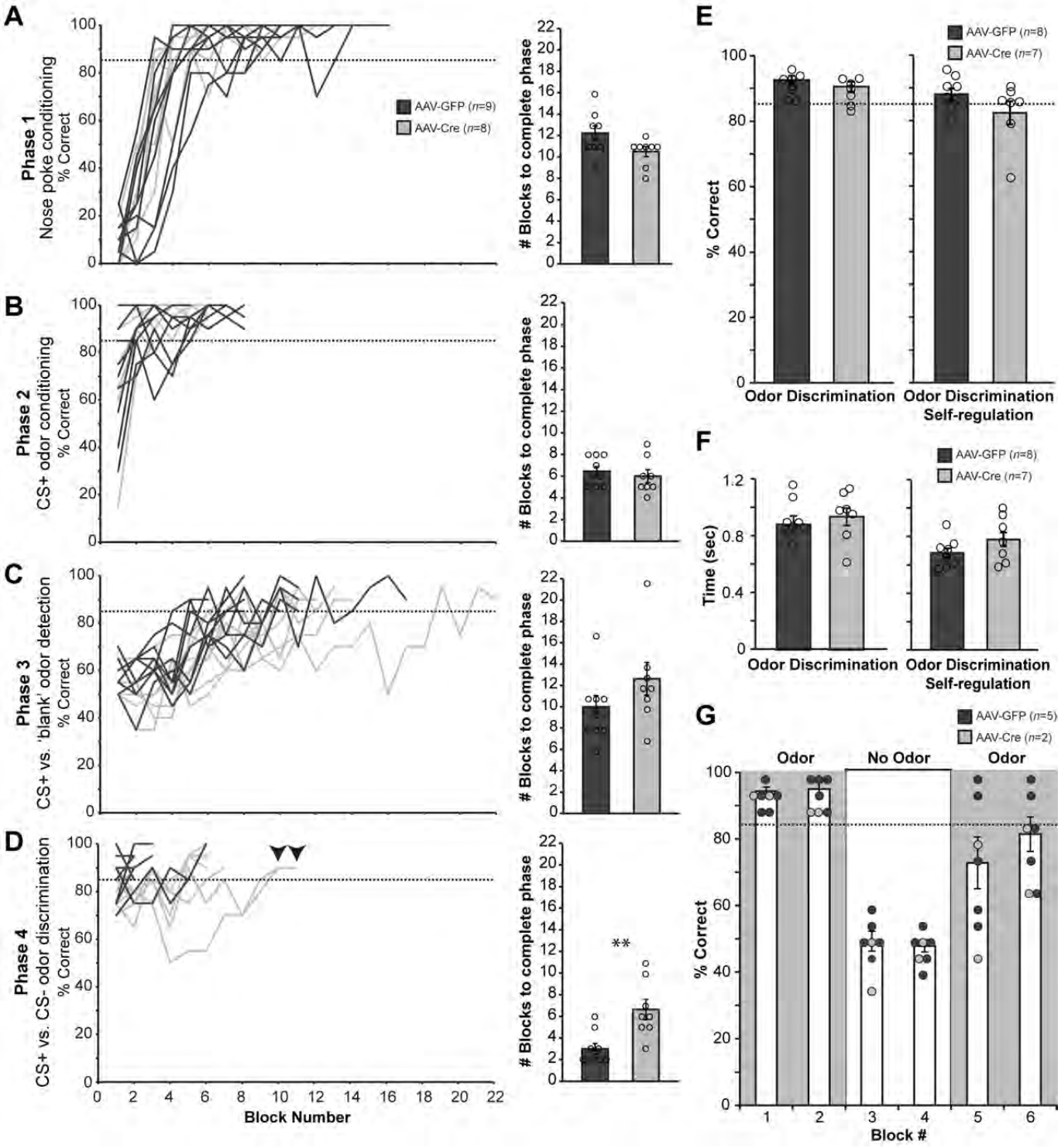

Figure 5

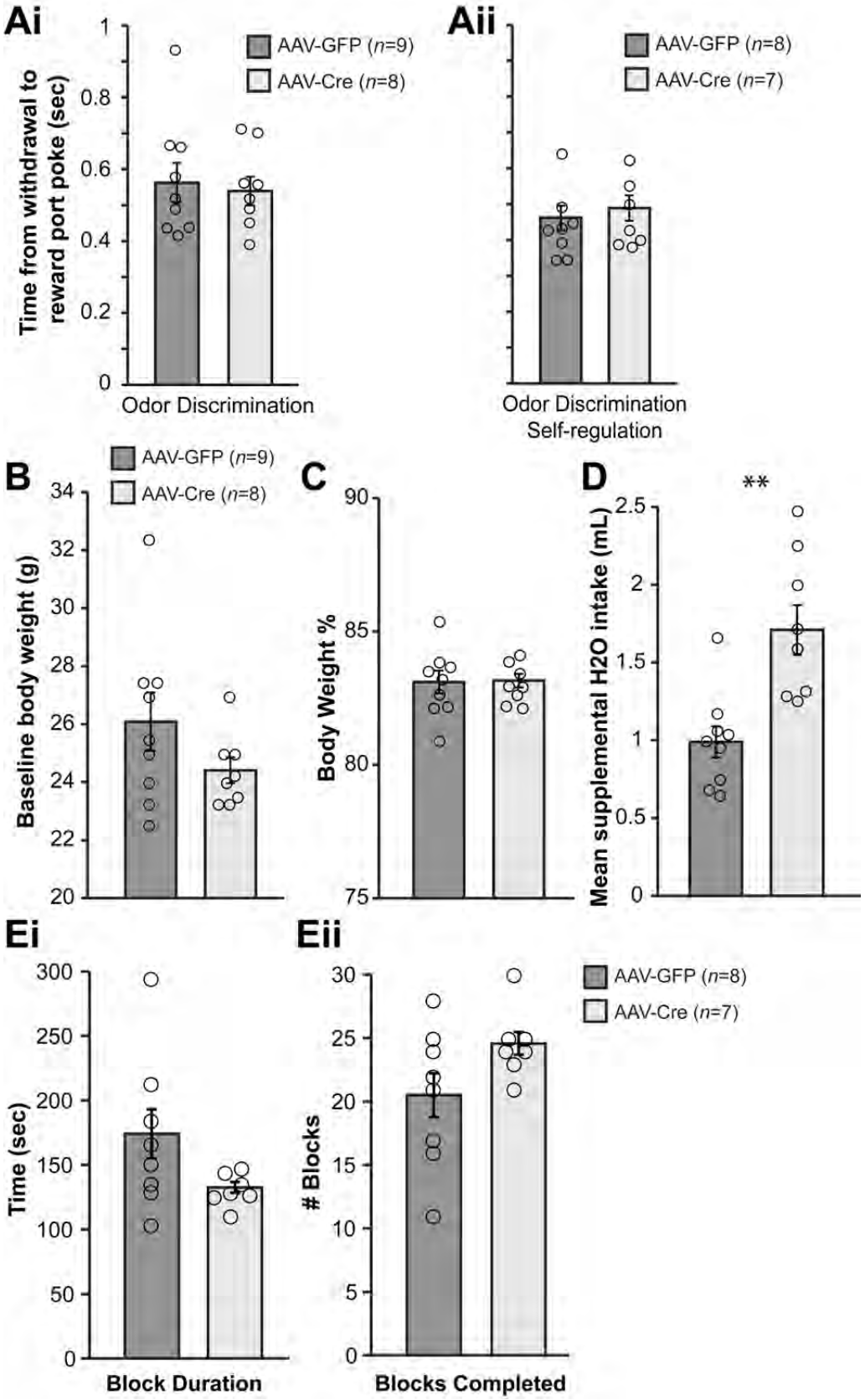

Figure 6

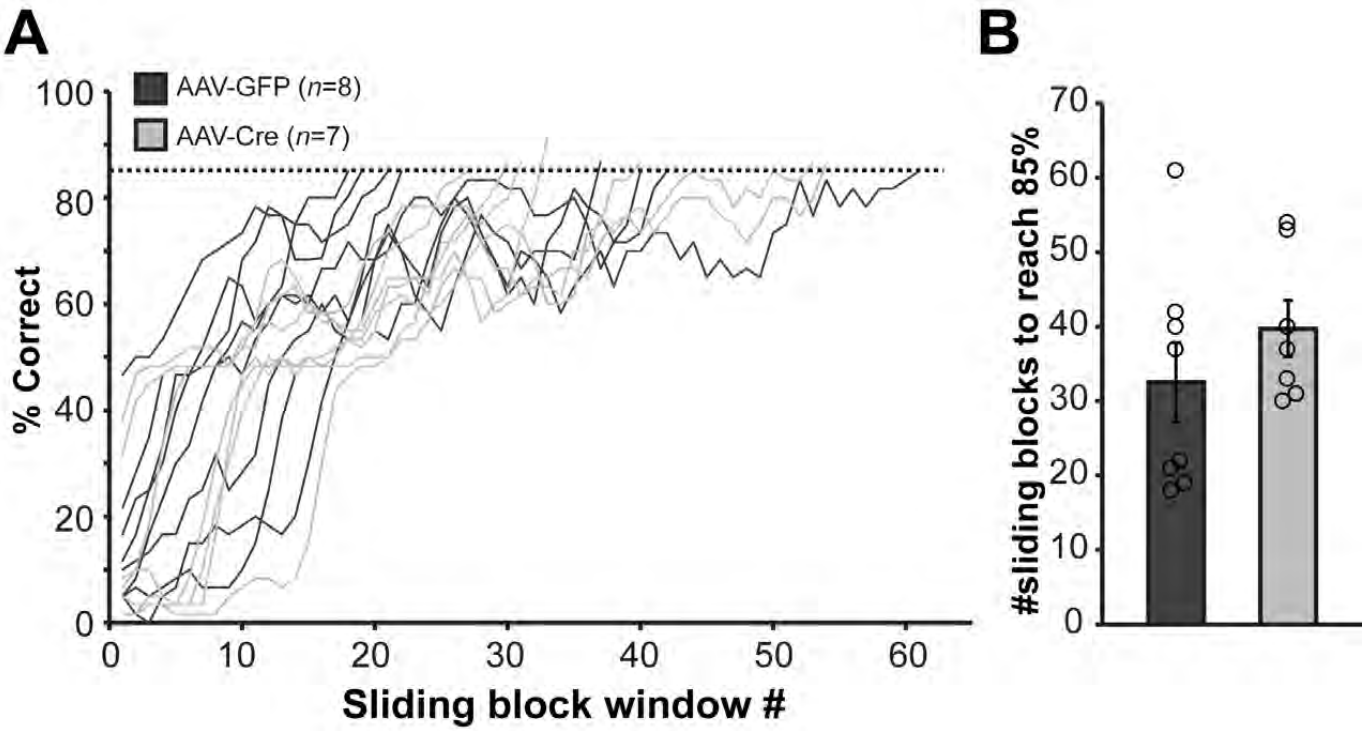

Figure 7