Serotonin 1A Receptors Alter Expression of Movement Representations

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Serotonin has a myriad of central functions involving mood, appetite, sleep, and memory and while its release within the spinal cord is particularly important for generating movement, the corresponding role on cortical movement representations (motor maps) is unknown. Using adult rats we determined that pharmacological depletion of serotonin (5-HT) via intracerebroventricular administration of 5,7 dihydroxytryptamine resulted in altered movements of the forelimb in a skilled reaching task as well as higher movement thresholds and smaller maps derived using high-resolution intracortical microstimulation (ICMS). We ruled out the possibility that reduced spinal cord excitability could account for the serotonin depletion-induced changes as we observed an enhanced Hoffman reflex (H-reflex), indicating a hyperexcitable spinal cord. Motor maps derived in 5-HT_{1A} receptor knock-out mice also showed higher movement thresholds and smaller maps compared with wild-type controls. Direct cortical application of the 5-HT_{1A/7} agonist 8-OH-DPAT lowered movement thresholds *in vivo* and increased map size in 5-HT-depleted rats. In rats, electrical stimulation of the dorsal raphe lowered movement thresholds and this effect could be blocked by direct cortical application of the 5-HT_{1A} antagonist WAY-100135, indicating that serotonin is primarily acting through the 5-HT_{1A} receptor. Next we developed a novel *in vitro* ICMS preparation that allowed us to track layer V pyramidal cell excitability. Bath application of WAY-100135 raised the ICMS current intensity to induce action potential firing whereas the agonist 8-OH-DPAT had the opposite effect. Together our results demonstrate that serotonin, acting through 5-HT_{1A} receptors, plays an excitatory role in forelimb motor map expression.

Introduction

Motor maps are topographical representations of movements in the motor cortex (Ferrier, 1873). When motor map integrity is disrupted, behavioral deficits are observed (Whishaw, 2000). Motor maps show plasticity and their expression changes following skilled motor learning and in neuropathophysiological conditions (Kleim et al., 1998, 2002, 2004). These experience-dependent changes to map expression are in part due to modulation of cortical synaptic connections and neuronal excitability (Hess and Donoghue, 1994; Monfils et al., 2004) particularly of layer V pyramidal neurons that make up the corticospinal tract

(Young et al., 2011). Enhancing cortical inhibition decreases motor map size and increases movement thresholds (Teskey et al., 2007; Young et al., 2011), whereas enhancing cortical excitability through elicitation of seizures has the opposite effect (Henderson et al., 2011). Previous studies have shown that several neuromodulators alter cortical motor map expression, including dopamine (Metz et al., 2004; Brown et al., 2009, 2011) and acetylcholine (Conner et al., 2003, 2010; Ramanathan et al., 2009).

Serotonin (5-hydroxytryptamine; 5-HT), a classic small molecule neurotransmitter, is synthesized in the raphe nuclei, which send widespread serotonergic projections throughout the brain and spinal cord (Moore et al., 1978; Bowker et al., 1983). Electrophysiological studies have demonstrated that bath application of serotonin to the isolated spinal cord causes fictive locomotion (Cazalets et al., 1992; Cowley and Schmidt, 1997; Hochman and Schmidt, 1998; Madriaga et al., 2004), indicating that serotonin is important for movement (Schmidt and Jordan, 2000). Surprisingly, the role of 5-HT on motor map expression has not been addressed, despite serotonin's fundamental and influential role on cortical mechanisms of movement (Vanderwolf, 1989) and its dense projections to the cortex from the median and dorsal raphe (Moore et al., 1978). A role in motor map expression would indicate a novel role for 5-HT in motor control beyond the direct raphe projections to the ventral horn of the spinal cord.

Postsynaptic serotonin signaling in the cortex relies primarily on inhibitory G-protein-coupled 5-HT_{1A} receptors (Barnes and

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Sharp, 1999); these are found on both GABAergic interneurons (Kruglikov and Bernardo, 2008) and layer V pyramidal cells (Puig et al., 2005), therefore calling for an empirical determination of serotonin's overall contribution to network excitability. To test the hypothesis that serotonin alters the expression of forelimb motor maps we used three approaches: (1) we interfered with 5-HT action, by depletion with intracerebroventricular injection of 5,7 dihydroxytryptamine (5,7 DHT), and local cortical application of the serotonin antagonist WAY-100135 in rats, and by using 5-HT_{1A}R knock-out (KO) mice; (2) serotonin release was activated within cortical networks by stimulation of the dorsal raphe and by local cortical application of the 5-HT_{1A/7} serotonin agonist 8-OH-DPAT; and (3) we observed separately the effects of 8-OH-DPAT and the 5-HT_{1A} antagonist WAY-100135 on electrophysiological properties of layer V pyramidal cells using a novel *in vitro* slice technique that replicates the essential methodological features of the intracortical microstimulation (ICMS) paradigm. Our results indicate that inhibition of 5-HT_{1A} receptor signaling suppresses motor map expression and has a deleterious effect on skilled motor behavior.

Materials and Methods

Rodents. Adult male Hooded Long–Evans (LE) rats ($n = 112$) and 5-HT_{1A} receptor KO ($n = 5$), heterozygous ($n = 5$), and wild-type (WT) mice ($n = 5$) were used in this study. All rats were obtained from Charles River. Mice were generated in a breeding colony maintained by Dr. Michael Antle at the University of Calgary from mice initially developed by Dr. Thomas Shenk (Princeton University, Princeton, NJ), and were bred on the C57BL/6J background. Information about the generation of the 5-HT_{1A} receptor KO mice can be found in Parks et al. (1998). The three genotypes of mice were produced by heterozygous $\times$ heterozygous crosses with two exceptions, two mice that came from a heterozygous $\times$ homozygous cross. All offspring were genotyped using a pair of KO primers (amplifying a 400 bp product that contains a piece of the neomycin cassette that replaced the start codon of the 5-HT_{1A} receptor gene) as well as a pair of WT primers (amplifying a 238 bp product that contains a segment of the 5-HT_{1A} receptor gene replaced by the neomycin cassette in the KO animals) to identify the presence or absence of each allele of the 5-HT_{1A} receptor gene (KO up primer: CTT TAC GGT ATC GCC GCT CCC GAT TC; KO down primer: TGC AGG ATG GAC GAA GTG CAG CAC A; WT up primer: AGTGCA GGC AGG CAT GGA TAT GTT; WT down primer: CCG ATGAGA TAG TTG GCA ACA TTC TGA; Smith et al., 2008).

Rats were housed individually and mice were group housed (up to five mice per cage) in clear plastic cages and were maintained on a 12 h light/dark cycle, lights on at 07:00 h, in separate colony rooms under specified pathogen-free conditions. Food and water were available *ad libitum*. All experimental procedures occurred during the light phase. Rodents were handled and maintained according to the Canadian Council for Animal Care guidelines. These procedures were approved by the Life and Environmental Sciences Animal Care and Health Sciences Animal Care Committees at the University of Calgary.

Drugs. All drugs were obtained through Sigma-Aldrich. Drugs for ICMS experiments were dissolved in physiological saline, whereas drugs for cell recordings were dissolved as stock solutions in distilled H₂O and stored at -20°C until use. 5,7 DHT creatinine sulfate salt was dissolved with 0.1% ascorbic acid. Desipramine hydrochloride was used at a dosage of 25 mg/kg and given in a volume of 1 ml/kg. The 5-HT_{1A} antagonist WAY-100135 maleate salt was used at a concentration of 6 μM (2 μl /injection site, total volume 6 μl) for ICMS procedures and 10 μM for bath application during cell recordings. The 5-HT_{1A/7} agonist (R)-(+)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) was used at a concentration of 80 μM (~ 0.5 ml to cover the ICMS window as needed) for ICMS procedures and 10 μM for bath application during cell recordings.

5-HT depletion surgery. To infer a role for serotonin in both forelimb motor map expression and learning to use the forelimb in a task requiring skill, we induced a widespread depletion of serotonin by injecting the

specific neurotoxin 5,7 DHT into a lateral ventricle according to the methodology of Hall et al. (1999). Thirty minutes before surgery, adult male LE rats received an intraperitoneal injection of desipramine hydrochloride (25 mg/kg) to protect the noradrenergic system. Rats were then anesthetized using isoflurane (5% induction). The incision site was shaved and the rat was placed into a stereotaxic apparatus with a flat skull position (incisor bar set 3.4 mm below the intra-aural plane). A subcutaneous injection of lidocaine was administered to the incision site 2 min before performing the incision. The connective tissue was removed from the skull and a small hole was drilled. To deplete serotonin rats received a 10 μl intracerebroventricular (anterior–posterior: $+0.8$ mm, mediolateral: $+1.5$ mm, dorsoventral: -3.5 mm, with respect to bregma) injection of 5,7 DHT (40 $\mu\text{g}/1$ μl of 0.1% ascorbic acid/physiological saline solution), using a 10 μl 30 gauge Hamilton microsyringe. The rate of infusion was 2 $\mu\text{l}/\text{min}$. The syringe was left in place for 2 min after the infusion to aid in diffusion, after which it was carefully removed. Bone wax was then used to occlude the drill hole. The scalp was then sutured and lidocaine jelly was applied to the incision site. Rats were then returned to their home cage.

HPLC in the forelimb motor neocortex. At the end of a two-week recovery period (control $n = 5$; lesion $n = 5$) we used HPLC to determine the extent to which intracerebroventricular administration of 5,7 DHT reduced serotonin in motor cortex. Rats were deeply anesthetized with sodium pentobarbital and were transcardially perfused with cold 0.1 M phosphate buffering saline (PBS). The brains were then extracted and the left motor cortex (~ 4 mm anterior and 3 mm posterior to bregma and 5 mm lateral to midline, encompassing the caudal forelimb area (CFA) as well as rostral forelimb area; RFA) was dissected and found to weigh between 93 and 135 mg. The dissected brain tissue was then placed in a polypropylene tube and placed on ice until it was used in HPLC analysis.

HPLC was performed according to the methodology of Horn et al. (1995). Ice-cold 0.2 M perchloric acid was used to homogenize the cortical tissue in a total volume of 500 μl (weight of cortex + volume of 0.2 M perchloric acid = 500 μl) for 30 s with an IKA Ultra Turrax homogenizer. The homogenate was centrifuged at 10,000 rpm at 4°C for 20 min. Aliquots of the supernatant (100 μl) were injected into a liquid chromatograph running a mobile phase of 100 mM ammonium dihydrogen phosphate, 2 mM sodium dodecylsulfate, 2 mM citric acid, 0.5 mM disodium EDTA in 90% water, and 10% methanol flowing at a rate of 1 ml/min over a Varian Micro-Pak C18–5 Column (4×100 mm, C-18 reverse phase, 5 μm particle size). A BAS LC-4b amperometric detector with a glassy carbon electrode held at $+0.75$ V was used to detect monoamines in the samples. A Waters Maxima data acquisition system was used to digitize and store the oxidation signal. The stored data were quantified using the external standard method, using fresh standards in 0.2 M perchloric acid. The detection limit was 1 pmol injected on column (Horn et al., 1995). Data were then converted to pmol/mg tissue by multiplying by five (to correct for HPLC tissue processing) and then dividing by the tissue weight.

Tryptophan hydroxylase (TPH) immunohistochemistry and cell counting in the dorsal and median raphe. We were also interested in determining the amount of cell loss in response to the intracerebroventricular administration of 5,7 DHT in the dorsal and median raphe, as both of these nuclei provide serotonergic projections to forebrain structures. Once the left motor cortex was dissected for HPLC, the remaining brain tissue was postfixed in 4% paraformaldehyde (PFA) for 48 h. The brains were then transferred to a 30% sucrose solution for 48 h for cryoprotection. An alternate series of 50 μm coronal sections were cut on a cryostat and collected throughout the rostrocaudal extent of the dorsal and median raphe. Sections were deposited into wells containing 0.1 M PBS with 0.02% sodium azide. Sections were rinsed in 0.5% hydrogen peroxide in PBSx (0.3% Triton X-100 in 0.1 M PBS) for 15 min to inactivate endogenous peroxidases. The sections were rinsed in PBSx, and were then blocked using 4% normal goat serum (NGS; Vector Laboratories) in PBSx for 90 min, after which the sections were incubated in a mouse anti-TPH primary antibody (1:5000; Sigma-Aldrich) in 1% NGS in PBSx for 48 h at 4°C on a shaker tray. Sections were rinsed again and then incubated in a biotinylated goat anti-mouse secondary antibody (1:200; Vector Laboratories) for 60 min. The sections were thoroughly rinsed

again and then incubated in an avidin-biotin complex (1:100; Vector Vectastain Elite ABC kit; Vector Laboratories) for 60 min, before being rinsed a final time, and reacted in a solution containing 24 ml of 0.1 M Tris buffer, 1 ml of 3,3'-diaminobenzidine tetrahydrochloride (DAB) solution (4 mg/ml, 0.4% solution), with 60 μ l of 8% nickel chloride to intensify the reaction product and 80 μ l of 30% hydrogen peroxide. The DAB reaction was quenched in a rapid series of PBSx rinses and sections were mounted on gelatin-coated slides, which were air dried and then dehydrated in alcohol rinses (70, 95, and 100% ethanol), cleared with xylene and coverslipped with Permount (Fisher Scientific).

Quantification was performed by counting the number of TPH-positive cells in the median and dorsal raphe nuclei and averaging over groups. Pictures of the raphe sections were taken with an Olympus BX51 microscope equipped with a QImaging QICAM 1394 camera using ImagePro Plus software (Media Cybernetics). Three (anterior, medial, and posterior) separate sections, 50 μ m each, through the raphe nuclei between 7.3 and 8.3 mm posterior from bregma were used for analysis for each rat. Cell counts from each of the three sections were performed using the cell counter plugin to ImageJ (National Institutes of Health). TPH-positive neurons were defined as being densely stained cell bodies that were visible on the sections.

Single pellet skilled reaching task. Previous research examining the effect of serotonin depletion on motor performance has found little, if any, effect on performance in sensorimotor tasks (Vanderwolf, 1989; Dringenberg et al., 1995; Gharbawie and Whishaw, 2003). To determine the effect of the serotonergic lesion on learning a task involving skilled use of the forelimb, we trained rats on the single pellet reaching task (Whishaw et al., 2003). This task requires learning to reach for a sugar pellet through a narrow slit in the apparatus. Rats were food restricted to 85–90% of free-feeding levels and given several banana flavored sucrose pellets (90 mg of Rodent Chow food pellets; Bioserve) before pretraining to ensure motivation and to familiarize them to the novel food. LE adult rats (control $n = 12$, lesion $n = 12$) were then pretrained in the single pellet skilled reaching task to determine handedness, and then received 5,7 DHT to the ventricle ipsilateral to the preferred forelimb to induce a serotonergic lesion. Reach training began 48 h after the lesion surgery and continued for a total of 10 daily sessions. We measured the number of reach attempts and percentage success on each training session. On the last session, the qualitative aspects of the 10 discrete subcomponents of the reaching behavior were also assessed. All apparatus, training methods, and analysis have been described previously (Whishaw et al., 2003; Henry et al., 2008).

ICMS. Standard high-resolution ICMS techniques were used to produce detailed threshold maps of forelimb regions of the motor cortex (Nudo et al., 1990; Kleim et al., 1998; Young et al., 2011). Twenty-four hours before surgery, rats were food deprived. Rats were initially anesthetized with intraperitoneal ketamine (100 mg/kg) and xylazine (5 mg/kg). Supplemental injections of either ketamine (25 mg/kg) or a mixture of ketamine (17 mg/kg) and xylazine (2 mg/kg) were given as needed to maintain a light anesthesia level. Mice were initially anesthetized with an intraperitoneal injection of ketamine (25 mg/kg) and xylazine (2.5 mg/kg). Supplemental injections of either ketamine (10 mg/kg) or a mixture of ketamine (10 mg/kg) and xylazine (1 mg/kg) were given as required to maintain a constant level of anesthesia. Level of anesthesia was monitored by assessment of breathing rate, whisker movements, and withdrawal reflex from a light pinch to the foot (rats) and tail (mice).

A craniotomy was performed to expose the motor cortex of the left hemisphere or the hemisphere contralateral to the preferred forelimb in the reaching experiment. The craniotomy extended ~4 mm anterior and 3 mm posterior from bregma, and extended ~5 mm lateral from the midline in rats. The craniotomy window in mice extended ~4 mm anterior and 3 mm posterior from bregma, and 3 mm lateral from midline. The cisterna magna was punctured with an 18-gauge needle to reduce brain swelling, and the dura matter was carefully removed from the neocortex. Silicon liquid warmed to body temperature (37–38°C) was placed on the exposed cortex, except when drugs were applied to the surface. A photograph magnified 32 $\times$ was taken using a digital camera connected to a stereomicroscope, which was then displayed on a personal computer. Using Canvas (version 11) imaging software (ACD Systems of

America), a grid consisting of 500 μ m squares was placed over the digital image. Electrode penetration occurred at the corners and middle of these squares unless there was a blood vessel, in which case no penetration occurred.

Electrodes pulled from glass capillary tubes were beveled and filled with 3.5 M sodium chloride and had impedance values of 1.0–1.5 M Ω . From the surface of the brain the electrode was lowered to a depth of 1550 μ m for rats and 800 μ m for mice, which corresponds to layer V of the cortex. Stimulation consisted of 13 monophasic 200 μ s cathodal pulses at 300 Hz (Young et al., 2011).

Rodents were maintained in the prone position, with their forelimb supported by a finger, elevating the limb for a closer inspection of possible movements. A threshold was determined at each penetration site. This was done by quickly increasing the current from 0 μ A toward 60 μ A until a movement was noted. The current was then decreased until the movement ceased. The movement threshold was defined as the minimal ICMS current able to elicit a movement. If the maximal current of 60 μ A was unable to elicit a movement, then the penetration site was considered nonresponsive.

First, the border of the motor map was defined with nonforelimb (hindlimb, jaw, neck, tail, trunk, and whisker) and nonresponsive points. Once the border was complete the forelimb (digit, wrist, elbow, shoulder) area was determined. Positive response sites were revisited during surgery to assay for changes in movement threshold as an indicator for anesthesia levels.

Motor maps were analyzed using Canvas software to calculate the areal extent of the RFA and CFA, respectively.

Spinal cord excitability at the forelimb level. Given that serotonin regulates spinal cord excitability and that a loss of function at the spinal cord level could account for our motor map findings, we examined the H-reflex using methods modified by Lee et al. (2005). Six (control $n = 3$; lesion $n = 3$) adult male LE rats were anesthetized by an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (5 mg/kg) and supplemented as needed with ketamine (25 mg/kg). Forelimbs were dissected to expose the median and ulnar nerves and isolate them from surrounding tissue to permit the placement of custom-made bipolar, silver stimulating hook electrodes under the nerves. Mineral oil was applied occasionally to prevent tissue desiccation. Acute electromyogram (EMG) recording electrodes (E2 subdermal needle electrodes; Grass Instrument Company) were inserted into the first dorsal interosseus. Bipolar stimulation was delivered by Clampex 10 via a Digidata 1440A (Molecular Devices) triggering a stimulation isolation unit (Stimulus Isolator A365; World Precision Instruments) connected to the bipolar stimulating electrode. Stimulation threshold (~50–75 μ A, 0.2 ms pulse duration) was measured for each animal by finding the minimum stimulation current sufficient to consistently evoke a short-latency, muscle compound action potential (AP; M-wave). After defining the stimulation threshold, the effects of various frequencies (0.1–2.0 Hz) and stimulation strengths (1.0–2.0 times threshold) on afferent-evoked muscle activity (H-reflex amplitude) were recorded to evaluate possible changes in spinal cord excitability caused by 5-HT depletion. The M-wave response was monitored during the H-reflex recording to confirm the stability and intensity of the electrical stimulation throughout the experiment. EMG recordings were amplified (10 $\times$ headstage, 200 $\times$ amplifier, 2000 $\times$ total) and filtered (low cut 300 Hz, high cut 3 kHz, EX4–400, Dagan) and then recorded and stored on PC for off-line analysis (10 kHz sampling rate, Clampex 10 and Clampfit; Molecular Devices). H-reflex amplitudes were measured from peak to peak.

Dorsal raphe electrode implantation surgery and stimulation parameters. Adult male LE hooded rats ($n = 8$) were anesthetized with 2–5% isoflurane. The heads were shaved and they were mounted in a stereotaxic frame. The skull was leveled between bregma and lambda. A twisted, Teflon-coated, bipolar electrode was chronically implanted into the dorsal raphe nucleus (DRN; coordinates: on midline, 7.3 mm posterior to and 6.0 mm ventral to bregma). The electrode assembly was anchored to the head using jeweler's screws and dental acrylic. ICMS was performed on the rats 1 week post-surgery.

Electrical current was delivered by a Grass S88x stimulator via a Grass SIU-C stimulus isolation unit (Astro-Med). Stimulation consisted of bi-

phasic 100 μ s square wave pulses that were delivered at 20 Hz. Dorsal raphe stimulation during ICMS was conducted at an intensity of 200 μ A for 90 s.

Rat ICMS with raphe stimulation and following 5-HT_{1A} receptor antagonist application. We electrically stimulated the dorsal raphe to directly observe the effect of serotonin release in the motor cortex on movement thresholds. Fifteen forelimb responsive sites, five nonresponsive and nonforelimb sites, were located for each rat. Movement thresholds were obtained three times at all electrode penetration sites. At each site, movement thresholds were taken before (prestimulation), during (stimulation), and after dorsal raphe stimulation (post stimulation). After the prestimulation movement threshold was determined, the dorsal raphe was stimulated for 90 s with the stimulation movement threshold taken within the last 10 s. The third movement threshold was taken 3 min following termination of dorsal raphe stimulation (poststimulation condition). Thus the movement threshold at each penetration point was derived three times over $\sim$ 5 min before moving on to the next point.

To determine whether the serotonin released in the motor cortex in response to dorsal raphe stimulation was acting through the 5-HT_{1A} receptor we applied the specific antagonist WAY-100135. Once all forelimb, nonforelimb responsive points and nonresponsive points had been determined using the ICMS technique, the 5-HT_{1A} receptor antagonist WAY-100135 (6 μ M solution in a volume of 2 μ l over 2 min per site; Lanfumey et al., 1993) or vehicle (physiological saline) was infused into the neocortex (layer V; 1.5 mm below cortex surface) at three sites: (1) medial, (2) anterior, (3) lateral to the forelimb responsive points. The needle was left in place for 2 min after the infusion. All ICMS sites were revisited twice. The thresholds for the prestimulation condition (with WAY-100135 or vehicle) were taken after the three injections of WAY-100135 or vehicle. After all the prestimulation thresholds were determined, the dorsal raphe was again stimulated for 90 s with the stimulation condition (with WAY-100135 or vehicle) thresholds taken within the last 10 s.

Histology. Immediately following ICMS rats were transcardially perfused with PBS and then with 4% PFA. The brains were then removed and postfixed in PFA for 24 h. The brains were cryoprotected by immersion in a 30% sucrose solution for 48 h. Slices (50 μ m) spanning the rostrocaudal extent of the dorsal and median raphe were collected using a cryostat and were stained with cresyl violet to determine electrode placement. Only rats that had the tip of the electrode in the DRN were used for analysis.

Rat ICMS with 5-HT_{1A/7} receptor agonist application. A final *in vivo* experiment was performed to determine whether the serotonin agonist 8-OH-DPAT, acting through the 5-HT_{1A} receptor also had the predicted effect of lowering ICMS-elicited movement thresholds. In adult male LE rats (control $n = 9$; lesion $n = 8$), ICMS was performed to derive a baseline motor map. Following the baseline ICMS mapping either 8-OH-DPAT solution (80 μ M in a volume of $\sim$ 0.5 ml of physiological saline) or vehicle was then applied directly to the surface of the neocortex. Thirty minutes later the motor cortex was remapped.

ICMS in slice electrophysiology. In the last two experiments we developed a novel *in vitro* slice technique that replicates the essential methodological features of the ICMS paradigm. We then observed separately the effects of 8-OH-DPAT and the 5-HT_{1A} antagonist WAY-100135 on the electrophysiological properties of layer V pyramidal cells subjected to slice ICMS. Seventeen male LE rats (postnatal day 34–39) were anesthetized using isoflurane and then decapitated. Using a methodology modified by Galic et al. (2008), the brain was quickly removed and placed in ice-cold slicing solution for several minutes containing the following (in mM): 87 NaCl, 2.5 KCl, 25 NaHCO₃, 0.5 CaCl₂, 7 MgCl₂, 1.25 NaH₂PO₄, 25 glucose, and 75 sucrose, saturated with 95% O₂/5% CO₂. The brain was then blocked and mounted on a vibratome (Leica) and submerged in ice-cold slicing solution. Coronal slices (300 μ m) containing motor cortex were taken from a region extending $\sim$ 600 μ m anterior and $\sim$ 300 μ m posterior to bregma. Slices were then incubated at 32°C for 30 min in artificial CSF (aCSF) containing the following (in mM): 126 NaCl, 2.5 KCl, 26 NaHCO₃, 2.5 CaCl₂, 1.5 MgCl₂, 1.25 NaH₂PO₄, and 10 glucose, saturated with 95% O₂/5% CO₂. Following incubation, slices were maintained in aCSF at room temperature (21–24°C) for a minimum of 30 min

before recording. Slices containing motor cortex were transferred to a recording chamber and superfused with 32°C aCSF at a flow rate of 1–2 ml/min.

Whole-cell recordings were obtained from layer V pyramidal cells visualized with an Axioskop II FS plus upright microscope (Zeiss) with a 40 $\times$ objective using infrared differential interference contrast optics. Layer V pyramidal cells were identified based on their tear-drop/triangular morphology with large apical dendrites orientated to the pial surface as well as electrophysiological characteristics (Bandrowski et al., 2003; Brill and Huguenard, 2010). Whole-cell recordings were obtained using borosilicate glass microelectrodes (tip resistance 3–5 M Ω) filled with a solution containing the following (in mM): 108 K-gluconate, 8 Na-gluconate, 2 MgCl₂, 8 KCl, 1 K₂-EGTA, 4 K₂-ATP, 0.3 Na₃ GTP, and 10 HEPES that was corrected to pH 7.25 with KOH. Membrane potentials were corrected for a liquid junction potential of 12 mV and subsequent voltages include liquid junction potential correction. Recordings were accepted for analysis if series resistance was <20 M Ω and changes in series resistance were $<20\%$ throughout the experiment. Electrophysiological signals were amplified using the Multiclamp 700A amplifier (Molecular Devices), low-pass filtered at 1 kHz, and digitized at 10 kHz using a Digidata 1322A (Molecular Devices). Data were collected (pClamp 9.0, Molecular Devices) and stored on a computer for offline analysis using Clampfit 9.0 (Molecular Devices).

Input resistance was measured as the slope of regression lines of plots of voltage responses to a family of 10 pA current steps. Intrinsic AP threshold was measured in response to a depolarizing current ramp and analyzed using Mini Analysis Software (Synaptosoft). Current-clamp recordings ($I = 0$) were used to assess layer V pyramidal cells' responses during stimulation of the slice, with parameters that are typical of the intracortical microstimulation used to assess movement representations in anesthetized rodents (Flynn et al., 2010; Brown et al., 2011; Tennant et al., 2011; Young et al., 2011). Slices were stimulated extracellularly with a patch pipette containing aCSF that was positioned directly dorsal ($\sim$ 130 μ m) to each layer V pyramidal cell body before recording from the latter in whole-cell configuration (preparation termed slice-ICMS). Electrical stimulation was delivered to the slice via an isolated stimulator (A-M Systems) and consisted of 13 monophasic cathodal pulses, each 200 μ s in duration, delivered at a frequency of 300 Hz, and repeated every second. Slice-ICMS current intensity was manually increased from 0 to 60 μ A, in 5 μ A increments each delivered for 10 s (i.e., each 5 μ A increment was administered for 10 1 Hz trains with each 1 Hz train consisting of 13 pulses). Pyramidal cell responses to slice-ICMS were assessed with four measures: (1) the resting membrane potential (RMP), (2) the minimum intensity of slice-ICMS current needed to evoke an AP (referred to as first AP discharge), (3) the percentage of cells exhibiting ≥ 1 AP at each slice-ICMS 5 μ A increment, and (4) the total number of APs at each slice-ICMS 5 μ A increment. All four slice-ICMS responses were assessed before and after bath application of either 8-OH-DPAT or WAY-100135.

Statistics. A Kruskal–Wallis test was conducted to assess treatment effects on cortical monoamine levels. Immunohistochemistry, behavior (percentage success and number of reach attempts), and H-reflex amplitudes were analyzed using separate independent samples *t* tests. Frequency amplitude modulation, reach attempts, and reaching success were each analyzed using a two-way ANOVA with Bonferroni *post hoc* comparisons. Independent samples *t* tests were used to compare movement thresholds between control and 5-HT-depleted rats, as well as control plus reach and 5-HT-depleted and reach-trained rats. To determine differences in motor maps between control, 5-HT-depleted, control and reach-trained, and 5-HT-depleted and reach-trained rats, a two-way ANOVA test with simple main effects was used. Map-remap data were analyzed using either a paired samples *t* test (map size) or the Wilcoxon signed ranks test (thresholds). A Wilcoxon matched-pair signed ranks test for planned comparisons with Bonferroni adjusted *p* values was used to compare thresholds taken during the prestimulation, stimulation, and stimulation + WAY-100135 conditions. An ANOVA test with a Tukey HSD *post hoc* was used to determine any differences in movement thresholds and motor maps between WT, heterozygous, and KO mice. For slice-ICMS, cell recordings were averaged across neurons for each condition. Significance was determined using a Student's *t* test or ANOVA

Table 1. Amount (pmol/mg) of five metabolites in the motor cortex

Treatment	5-HT	5-HIAA	NE	DA	DOPAC
Control	1.06 ± 0.21	0.25 ± 0.17	1.66 ± 0.31	0.22 ± 0.11	0.50 ± 0.17
5,7 DHT	0.00*	0.13 ± 0.13	1.13 ± 0.25	0.12 ± 0.03	0.37 ± 0.11

Asterisk (*) indicates being significantly different from control at $p < 0.05$. 5-HIAA, 5-Hydroxyindoleacetic acid; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; NE, norepinephrine.

with *post hoc* comparisons based on what was appropriate for the number of conditions and/or measurements. A paired two-tail Student's *t* test was used to assess differences in intrinsic membrane properties. A Wilcoxon signed rank one-tail test was used to assess differences in the number of APs elicited at each 5 μ A current increment for slice-ICMS. An α value of 0.05 was used in all experiments and all statistical analyses were conducted using Statistical Package for the Social Sciences (IBM SPSS). Data are presented as mean $\pm$ SEM.

Results

5,7 DHT abolishes 5-HT in the rat forelimb motor neocortex and reduced TPH-positive cells in the dorsal and median raphe

There was no detectable serotonin in the motor cortex 2 weeks following 5,7 DHT-treatment ($\chi^2(1) = 7.76$; $p = 0.005$). No significant differences were found for the amount of 5-hydroxyindoleacetic acid, norepinephrine, dopamine (DA) or 3,4-dihydroxyphenylacetic acid between 5-HT-depleted and control rats ($p > 0.05$; Table 1).

TPH immunohistochemistry revealed that the serotonin depletion caused a significant loss of TPH-positive cell bodies in the dorsal (39%) and median (35%) raphe compared with control rats. The total number of TPH-positive cells in the dorsal raphe was significantly lower ($t_{(8)} = 7.41$, $p = 0.001$) in the 5-HT-depleted rats (509 ± 38) compared with the control rats (835 ± 22). The total number of TPH-positive cells in the median raphe was also significantly lower ($t_{(8)} = 4.08$, $p = 0.004$) in the 5-HT-depleted rats (115 ± 7) compared with the control-treated rats (176 ± 13).

Serotonin depletion produces higher movement thresholds and smaller motor maps in rats

The total forelimb movement thresholds were significantly higher in nonreach-trained 5-HT-depleted ($37.37 \pm 1.22 \mu$ A) rats compared with control nonreach-trained ($28.60 \pm 1.24 \mu$ A) rats ($t_{(13)} = 5.01$, $p = 0.001$; Fig. 1). There were no significant differences in total forelimb movement thresholds between control and reach-trained ($30.40 \pm 1.52 \mu$ A) and 5-HT-depleted and reach-trained ($31.38 \pm 1.25 \mu$ A) rats. There were main effects of serotonin depletion ($F_{(1,35)} = 27.75$, $p < 0.001$) and reach training ($F_{(1,35)} = 51.31$, $p < 0.001$); however, there was no significant interaction between serotonin depletion and reach training ($F_{(1,35)} = 0.01$, $p = 0.92$). An analysis of the main effects showed that reach training significantly increased motor map size in control rats ($F_{(1,35)} = 8.42$, $p = 0.006$) as well as in 5-HT-depleted rats ($F_{(1,35)} = 10.05$, $p = 0.003$). No significant differences were found in the amount of ketamine ($F_{(3,35)} = 0.55$, $p = 0.65$) or xylazine ($F_{(3,35)} = 1.98$, $p = 0.14$) as a function of body weight and duration of surgery between groups, indicating that changes in movement thresholds and map size were not a function of anesthesia depth.

Serotonin depletion results in behavioral impairments in skilled reaching

Both the 5-HT-depleted and control rats showed a significant increase in reach attempts over the 10 training days ($F_{(9,110)} =$

Figure 1. Serotonin depletion decreases and reach training increases motor maps. Coded forelimb movement representations of the left motor cortex of control, control and reach-trained, 5-HT-depleted, and 5-HT-depleted and reach-trained rats derived with ICMS. Map borders are defined as electrode penetration sites where a maximum current of 60 μ A did not elicit forelimb movements. Quantification of ICMS derived total motor map size from control, control and reach-trained, 5-HT-depleted, and 5-HT-depleted and reach-trained rats. Reach training in 5-HT-depleted rats returned maps size to control (nonreach-trained) levels. Significance was indicated when $*p < 0.05$. Error bars indicate SEM. A, anterior; M, medial; P, posterior; L, lateral.

15.22, $p = 0.0001$) and while there were significant differences between 5-HT-depleted and control rats ($F_{(1,9)} = 10.84$, $p = 0.001$), *post hoc* follow-up tests revealed that the 5-HT-depleted rats only had fewer reach attempts on the first 3 d of training. The interaction between day and group was not significant ($F_{(9,110)} = 0.47$, $p = 0.89$). With respect to the percentage reaching success, again both groups showed a significant increase over the 10 training days ($F_{(9,110)} = 4.45$, $p = 0.0001$) but the 5-HT-depleted and control rats did not significantly differ in their percentage success ($F_{(1,9)} = 2.46$, $p = 0.37$). However, a frame-by-frame analysis of forelimb movements revealed that 5-HT-depleted rats had significantly greater error scores on 3 of the 10 reaching components relative to controls. In 5-HT-depleted rats, the mean error score for component 3, elbow to midline (0.23 ± 0.10); component 8, supination I (0.18 ± 0.07); and component 9, supination II (0.37 ± 0.14) were significantly higher compared with control rats' mean error scores for components 3 (0.03 ± 0.03), 8 (0.00 ± 0.00), and 9 (0.03 ± 0.03) ($z = -2.22$, $p = 0.023$; $z = -2.40$, $p = 0.030$; $z = -2.26$, $p = 0.023$, respectively). In control rats, component 3, elbow to midline, is performed by the elbow being adducted to the midline while the tips of the digits retain their alignment with the midline of the body. In 5-HT-depleted rats

the movement was missing or the rats did not fully adduct their elbow to midline. In control rats, component 8, supination I, as the limb is being withdrawn, the hand supinates 90°; however, in 5-HT-depleted rats this movement was missing or the rats did not supinate their hand to the full 90°. In control rats, component 9, supination II, once the hand is withdrawn from the slot and raised to the mouth, the hand further supinates by ~45° to place the food in the mouth. 5-HT-depleted rats also either did not make this movement or they did not fully supinate to 45°. These modest effects on behavior following serotonin depletion are consistent with previous observations by (Vanderwolf, 1989; Gharbawie and Whishaw, 2003). Moreover, the alterations in a few forelimb movements are also consistent with previous reports following mild lesions of the forelimb motor map area (Gharbawie et al., 2005).

Spinal cord excitability at the forelimb level is not reduced following 5-HT depletion

Since rats with serotonin depletion exhibited higher movement thresholds and smaller motor maps, we wanted to determine whether this was caused by a hypo-excitability spinal cord. 5-HT-depleted rats had significantly higher H-reflex amplitudes compared with control rats ($t_{(74)} = 2.31$, $p = 0.02$; Fig. 2*A,B*). The normalized H-reflexes of lesioned and control rats exhibited a significant ($F_{(1,6)} = 30.91$, $p < 0.0001$) frequency-dependent modulation. At both 1 and 2 Hz the normalized H-reflex of 5-HT-depleted rats was significantly larger compared with control rats ($p < 0.001$, both cases); lesioned rats had H-reflex facilitation relative to control rats (Fig. 2*C*). Given that changes in the spinal cord were unlikely to be responsible for the motor map and skilled reaching alterations brought about by serotonin depletion, we next asked which 5-HT receptor was involved in 5-HT action in the cortex.

Activation of 5-HT_{1A} receptor results in lower movement thresholds and larger motor maps in rats

Cortical application of 8-OH-DPAT significantly ($z = -2.03$, $p = 0.04$) lowered forelimb movement thresholds (Control = $36.23 \pm 1.73 \mu\text{A}$; 8-OH-DPAT = $31.07 \pm 2.32 \mu\text{A}$). No significant ($z = -0.46$, $p = 0.65$) differences were found between presaline movement thresholds ($32.21 \pm 1.96 \mu\text{A}$) and post saline movement thresholds ($31.17 \pm 1.57 \mu\text{A}$).

With respect to total forelimb map size in control rats, saline did not have a significant ($t_{(4)} = 1.29$, $p = 0.27$) effect (presaline = $6.48 \pm 0.71 \text{ mm}^2$ and post saline = $6.88 \pm 0.73 \text{ mm}^2$). However, application of 8-OH-DPAT to the motor cortex produced larger forelimb motor maps (Fig. 3).

Control rats that received 8-OH-DPAT had significantly larger total forelimb map area compared with their pre-8-OH-DPAT total forelimb map area ($t_{(3)} = 9.70$, $p = 0.002$). Similarly 8-OH-DPAT-induced motor map changes were found in 5-HT-depleted rats. In 5-HT-depleted rats the presaline total forelimb map size ($4.28 \pm 0.51 \text{ mm}^2$) was also not significantly different from the post saline map size ($4.68 \pm 0.63 \text{ mm}^2$; $t_{(4)} = 1.75$, $p = 0.16$). However, 5-HT-depleted rats that received 8-OH-DPAT had significantly larger total forelimb map area compared with their pre-8-OH-DPAT (Fig. 3) total forelimb map area ($t_{(2)} = 4.85$, $p = 0.04$).

No significant differences were found in the amount of ketamine ($t_{(15)} = 0.13$, $p = 0.90$) or xylazine ($t_{(15)} = 0.20$, $p = 0.84$) as a function of body weight and duration of surgery between rats that received saline and rats that received 8-OH-DPAT before deriving the second forelimb motor map, indicating that changes

Figure 2. 5-HT depletion increased spinal reflex excitability. 5,7 DHT lesions increased H-reflex amplitude and switched frequency-dependent depression into facilitation. *A*, Examples of averaged (10 sweeps) H-reflex recordings from control (left) and lesioned (right) animals. Scale bar, 25 μV /2 ms. *B*, Mean H-reflex amplitudes recorded at 0.1 Hz, pooled from 1.0 to $2.0 \times$ threshold, was significantly larger in lesioned ($262.8 \pm 47.5 \text{ mV}$) compared with control rats ($149.2 \pm 25.2 \text{ mV}$, $p < 0.05$). *C*, The normalized H-reflexes of 5-HT-depleted (triangles) and control rats (circles) exhibited frequency-dependent modulation. Significance was indicated when $p < 0.05$. Error bars indicate SEM.

in movement thresholds and map size were not a function of anesthesia depth. Overall our results indicate that serotonin increases motor map size in both control and 5-HT-depleted rats. To further explore the role of the serotonin 1A receptor we mapped 5-HT_{1A} KO mice.

5-HT_{1A} receptor KO mice have smaller motor maps

There was a significant effect of group on all forelimb movement thresholds ($F_{(2,12)} = 5.35$, $p = 0.02$). 5-HT_{1A} receptor KO mice had significantly higher movement thresholds ($42.37 \pm 3.12 \mu\text{A}$, $p = 0.018$) compared with heterozygous mice ($28.80 \pm 3.28 \mu\text{A}$), but not WT mice ($34.45 \pm 2.36 \mu\text{A}$, $p = 0.18$).

There was a significant effect of group on motor map size ($F_{(2,12)} = 5.91$, $p = 0.016$). 5-HT_{1A} receptor KO mice had significantly smaller total forelimb map area ($p = 0.013$) compared with WT, but were not significantly different from heterozygous mice (Fig. 4) ($p = 0.21$).

No significant differences were found in the amount of ketamine ($F_{(2,12)} = 0.31$, $p = 0.74$) or xylazine ($F_{(2,12)} = 0.23$, $p = 0.80$) as a function of body weight and duration of surgery between groups, indicating that changes in movement thresholds and map size were not a function of anesthesia depth.

Figure 3. 5-HT_{1A/7} receptor agonist (8-OH-DPAT) increases motor map size. Coded forelimb movement representations of the left motor cortex of control, control and 8-OH-DPAT, 5-HT-depleted, and 5-HT-depleted and 8-OH-DPAT rats derived with ICMS. Map borders are defined as electrode penetration sites where a maximum current of 60 μ A did not produce a forelimb movement. Quantification of total motor map size increased post-8-OH-DPAT in both control and 5-HT-depleted rats. Significance was indicated when $*p < 0.05$. Error bars indicate SEM. M, medial; P, posterior; A, anterior; L, lateral.

DRN stimulation lowers and WAY-100135 increases movement thresholds in rats

As an alternate way to elevate serotonin in the cortex we stimulated the dorsal raphe during the motor map procedure and then, to determine whether it was a serotonin-mediated effect, we blocked the raphe stimulation-induced lowering of movement thresholds with the specific serotonin 1A antagonist WAY-100135.

Movement thresholds during DRN stimulation ($16.85 \pm 0.91 \mu$ A) were significantly lower compared with prestimulation levels ($24.74 \pm 1.21 \mu$ A; $Z = -6.74$, $p = 0.001$), indicating an excitatory effect of DRN stimulation. During the poststimulation phase movement thresholds returned to prestimulation levels and were significantly higher than during DRN stimulation ($23.55 \pm 0.87 \mu$ A; $Z = 6.59$, $p = 0.001$). This DRN stimulation

Figure 4. 5-HT_{1A} receptor KO mice have smaller motor maps. Coded forelimb movement representations of the left motor cortex of WT, heterozygous (HET), and 5-HT_{1A} receptor KO mice, derived with ICMS. Map borders are defined as electrode penetration sites where a maximum current of 60 μ A did not produce a forelimb movement. Quantification of ICMS motor map size from WT, HET, and 5-HT_{1A} receptor KO mice showed that 5-HT_{1A} receptor KO mice had significantly smaller total map sizes compared with WT mice. Significance was indicated when $*p < 0.05$. Error bars indicate SEM. M, medial; P, posterior; A, anterior; L, lateral.

effect could not be accounted for by the brief 5 min interval between deriving the first, second, and third movement threshold at each penetration point as movement thresholds are stable over hours (Nudo et al., 1990).

Movement thresholds were not affected by vehicle (0.9% saline) application to the neocortex, but WAY-100135 during DRN stimulation resulted in significantly higher movement thresholds ($21.85 \pm 1.29 \mu$ A) compared with stimulation alone ($16.85 \pm 0.91 \mu$ A; $Z = -2.80$, $p = 0.005$), indicating that the movement threshold-lowering effect of DRN stimulation was mediated by 5-HT_{1A}Rs.

WAY-100135 decreases ICMS-induced excitation of layer V pyramidal neurons

The *in vivo* ICMS motor map data thus far point to serotonin exerting an overall local excitatory effect on motor cortex via

Figure 5. 5-HT_{1A} antagonist WAY-100135 decreases ICMS-induced excitation of layer V pyramidal neurons. In coronal slices of motor cortex ICMS stimulation was administered with an extracellular electrode (slice-ICMS) while individual layer V pyramidal neurons were recorded in a whole-cell configuration. **A**, Two representative current-clamp traces showing the responses of a layer V pyramidal cell before (Baseline) and after (WAY-100135) bath application of WAY-100135 (10 μ M). ICMS current intensity was increased from 0 to 60 μ A in 5 μ A increments with 10 s trains at each increment (see Materials and Methods). Small vertical excursions are stimulation artifacts. **B**, Percentage of cells firing at each 5 μ A increment of ICMS stimulation. **C**, Mean total number of APs evoked at each 5 μ A increment of ICMS stimulation. **D**, The mean minimum of ICMS stimulation to first elicit an AP discharge ($n = 8$). **E**, Mean RMP measured directly before administering ICMS stimulation to the slice. Significance was indicated when $*p < 0.05$. Error bars indicate SEM.

5-HT_{1A}Rs. To examine the possibility that serotonin is exerting this excitatory effect by contributing to the excitability of pyramidal cells from motor cortex a brain slice preparation was used. In this preparation, extracellular ICMS stimulation was applied to slices of motor cortex while whole-cell patch recordings were performed on pyramidal cells from the output layer of this brain region. Individual layer V pyramidal cells consistently responded to ICMS stimulation by stereotypical patterns of somatic depolarization of membrane potential and APs with increasing ICMS current intensities from 5 to 60 μ A. Somatic depolarization occurred <10 ms following the initiation of each ICMS train whereas APs were first observed with a mean latency of 19.0 ms. Pharmacological modulation of serotonin receptors with the 5-HT_{1A} antagonist WAY-100135 (10 μ M) reduced excitation of layer V pyramidal cells in response to ICMS stimulation (Fig. 5A; $n = 8$ cells). The number of cells responding with APs (minimum 1 AP per 5 μ A increment) was reduced following WAY-100135 (Fig. 5B). Cells exhibited a significantly lower number of APs following application of WAY-100135 at current increments of 30, 35, 40, and 45 μ A ($p < 0.05$ all cases; Fig. 5C). The minimum ICMS current required to elicit the first AP was significantly increased with WAY-100135 ($t_{(7)} = 2.14$, $p = 0.04$ Fig. 5D). There was a trend for RMP to be hyperpolarized following application of

WAY-100135 when RMP was assessed immediately before ICMS stimulation ($t_{(7)} = 1.55$, $p = 0.08$; Fig. 5E). The input resistance of pyramidal cells was not significantly affected by WAY-100135 (Pre-WAY-100135: 79 ± 8 M Ω , Post-WAY-100135: 85 ± 7 M Ω ; $t_{(7)} = 1.44$, $p = 0.19$). The AP threshold of pyramidal cells was not significantly affected by WAY-100135 (Pre-WAY: 39 ± 0.4 mV, Post-WAY: 39 ± 0.8 mV; $t_{(7)} = 0.83$, $p = 0.43$).

8-OH-DPAT increases ICMS-induced excitation of layer V pyramidal neurons

Application of the 5-HT_{1A/7} agonist 8-OH-DPAT (10 μ M) increased excitation of layer V pyramidal cells in response to ICMS stimulation (Fig. 6A; $n = 9$ cells). The number of cells responding with APs (minimum 1 per 5 μ A increment) was increased following 8-OH-DPAT (Fig. 6B). Cells exhibited a significantly higher number of APs following application of 8-OH-DPAT at ICMS current increments of 20, 25, and 30 μ A ($p < 0.05$ all cases; Fig. 6C). The minimum 5 μ A increment of ICMS current required to elicit the first AP was significantly decreased with 8-OH-DPAT ($t_{(8)} = 8.00$, $p = 0.0001$ Fig. 6D). RMP assessed immediately before ICMS stimulation was significantly depolarized following application of 8-OH-DPAT ($t_{(8)} = 1.84$, $p = 0.05$; Fig. 6E). The input resistance of pyramidal cells was not significantly affected by 8-OH-DPAT (Pre-OH-DPAT: 90 ± 8 M Ω , Post-OH-DPAT: 87 ± 6 M Ω ; $t_{(7)} = 0.64$, $p = 0.54$). The AP threshold of pyramidal cells was not significantly affected by 8-OH-DPAT (Pre-OH-DPAT: 38 ± 1 mV, Post-OH-DPAT: 39 ± 1 mV; $t_{(7)} = 1.03$, $p = 0.34$).

Discussion

This study is the first demonstration of a role for serotonin in the expression of motor maps. In rats, 5,7 DHT treatment that resulted in undetectable levels of serotonin in the motor cortex raised ICMS movement thresholds and decreased motor map size relative to appropriate controls. 5-HT-depleted rats also displayed errors in forelimb movements during skilled reaching. Subsequent experiments here indicate that 5-HT_{1A}Rs contribute to serotonin's effect on motor maps by demonstrating directional excitability changes during genetic or pharmacological manipulation of these receptors.

5-HT_{1A}R KO mice had smaller motor map areas compared with WT controls. Direct application of the 5-HT_{1A/7} agonist 8-OH-DPAT to motor cortex increased motor map expression in both naive rats and 5-HT-depleted rats. In naive rats, stimulation of the DRNs lowered ICMS movement thresholds, and this effect was blocked by the 5-HT_{1A}R antagonist WAY-100135. Based on these *in vivo* data, a slice preparation was developed to examine how manipulation of 5-HT_{1A}R activity during ICMS stimulation affected the excitability of layer V pyramidal neurons that make up the principle output layer of sensorimotor cortex. ICMS stim-

ulation delivered extracellularly to slices of motor cortex resulted in membrane depolarization of layer V pyramidal cells followed by AP firing as stimulation intensities were increased. The ICMS elicited AP responses were decreased with WAY-100135 and increased with 8-OH-DPAT. Collectively, these data suggest that 5-HT, acting through 5HT_{1A}Rs, has an overall excitatory effect on the neural networks that give rise to movement representations.

5-HT-depleted rats showed increased errors on three subcomponents of forelimb movements during skilled reaching behavior. While the relationship between cortical movement representations and behavior is not simple (Boychuk et al., 2011), decreases or increases in motor map size are often accompanied by deficits in fine motor control. Abnormal motor maps and decreased skilled reaching performance have previously been observed following cortical ischemia (Kleim et al., 2003), repeated seizures (Henry et al., 2008), DA depletion (Brown et al., 2011), and high-frequency stimulation intended to saturate cortical excitability (Henderson et al., 2012). While the 5-HT-depleted rats were able to learn the skilled motor task, the way in which they performed the task was altered. Together, these findings suggest that perturbations in motor maps reflect abnormalities in cortical function that contribute to behavioral deficits. It is possible that some of serotonin's contribution to motor map expression *in vivo* is mediated indirectly by its effect on levels of other neurotransmitters in the brain. For example, previous research has shown that serotonin affects the release of DA as well as acetylcholine (Nakai et al., 1998; Fink and Göthert, 2007); it is therefore possible that serotonin *in vivo* modulates available DA and acetylcholine in subcortical structures and that this could contribute to the motor map changes we observed. The changes in motor maps and behavior with serotonin depletion in this study may also in part be mediated by structures downstream of cortex, as previous studies have shown that the basal ganglia, brainstem, and cerebellum are also used in skilled motor learning and task performance (Whishaw et al., 1992, 1993, 2007). In particular, serotonin depletion may also alter spinal cord circuits that rely on serotonin. Serotonin receptors are found in the spinal cord (Daval et al., 1987; Croul et al., 1998) and serotonin modulates spinal cord reflex circuitry (Jackson and White, 1990; Elliott and Wallis, 1992; White et al., 1996; Hedo et al., 2002; Honda et al., 2004). To examine this possibility, the H-reflex was measured in 5-HT-depleted rats to assess the monosynaptic activation of α motor neurons by group 1a afferents (Ho and Waite, 2002; Hosoido et al., 2009). We observed greater H-reflex amplitudes at the level of the forelimb in 5-HT-depleted rats compared with controls. Moreover serotonin depletion caused the spinal cord to exhibit frequency-dependent facilitation, instead of depression, indicating that serotonin depletion has an effect at the level of the spinal cord.

Figure 6. 5-HT_{1A/7} agonist 8-OH-DPAT increases ICMS-induced excitation of layer V pyramidal neurons. In coronal slices of motor cortex ICMS stimulation was administered with an extracellular electrode (slice-ICMS) while individual layer V pyramidal neurons were recorded in a whole-cell configuration. **A**, Two representative current-clamp traces showing the responses of a layer V pyramidal cell before (Baseline) and after (8-OH-DPAT) bath application of 8-OH-DPAT (10 μ M) during increasing ICMS current intensities from 0 to 60 μ A in 5 μ A increments. **B**, Percentage of cells firing at each 5 μ A increment of ICMS stimulation. **C**, Mean total number of APs evoked at each 5 μ A increment of ICMS stimulation. **D**, The mean minimum of ICMS stimulation to first elicit an AP discharge ($n = 9$). **E**, Mean resting membrane potential measured directly before administering ICMS stimulation to the slice. Significance was indicated when $*p < 0.05$. Error bars indicate SEM.

One possibility for this change in the H-reflex is an increase in sensory transmission via removal of inhibition of the reflex, while another is an increase in motor neuron excitability as serotonin depletion has previously been associated with decreased motor neuron excitability (Pflieger et al., 2002). These findings suggest that the raised ICMS thresholds and decreased motor map size cannot be accounted for simply by a loss of excitability of spinal circuits following serotonin depletion.

The present results suggest that serotonin exerts an overall direct excitatory effect on cortical motor maps that is mediated by the activity of 5-HT_{1A}Rs. Here, cortical application of 8-OH-DPAT *in vivo* increased motor map size and decreased ICMS thresholds to evoke movement, whereas application of WAY-100135 increased thresholds. Motor maps of 5-HT_{1A}R KO mice were smaller than WT controls. While developmental compensation is always a concern with KO animal models, this is likely a limited concern in the present study due to the concordance between the KO and antagonist findings. To further explore 5-HT-dependent changes of excitability in motor cortex, this study then used brain slices from the motor cortex to record from individual layer V pyramidal cells during ICMS stimulation. Moreover anesthetics are not used in the slice-ICMS preparation and therefore their potential confounding influence is removed. Since initial brain mapping studies over a century ago (Fritsch

and Hitzig, 1870), work has centered on understanding how these motor maps relate to cortical circuitry and excitability (Graziano et al., 2002; Strick, 2002; Rathelot and Strick, 2006). Based on *in vivo* measurements of axon volley latencies, ICMS techniques that produce motor maps rely on direct and indirect (*trans*-synaptic) activation of pyramidal cells (Jankowska et al., 1975; Bolay and Dalkara, 1998). While our understanding of the brain activation responses to cortical microstimulation continues to grow, how many of the neurotransmitter systems affect motor maps is still unknown. Here, somatic whole-cell patch recordings during ICMS stimulation revealed a small set of consistent activation responses in these cells. These responses occurred in close temporal relation to the electrical ICMS train and included membrane depolarization beginning <10 ms from the start of the stimulation and AP firing with increasing stimulation intensities (mean latency to fire at threshold: 19.0 ms). Importantly, these slice-ICMS AP responses appear relevant to motor maps as both the *in vitro* and *in vivo* responses exhibited heightened excitability with a 5-HT_{1A}R agonist and reduced excitability with a 5-HT_{1A}R antagonist. In the slice-ICMS preparation WAY-100135 decreased both the number of pyramidal cells firing APs and the total number of AP responses while increasing the stimulation intensities required to first elicit APs. Application of 8-OH-DPAT resulted in the opposite effect on these measures indicating that pyramidal cell excitability increased with 8-OH-DPAT. The striking similarity between movement thresholds and slice-ICMS AP responses during pharmacological manipulation of 5-HT_{1A}Rs supports that *in vivo* motor maps are heavily influenced by the excitability of layer V pyramidal cells.

Serotonin's overall excitatory effect on cortex involves a positive shift in the proportion of glutamatergic to GABAergic signaling onto pyramidal cell networks in cortex. Bath application of 5-HT increases the excitatory drive onto layer V pyramidal cells in frontal cortex by increasing frequency and amplitude of sEPSCs (Lambe et al., 2000). Fast spiking GABAergic interneurons have been shown to be involved in feedforward inhibition of pyramidal neurons (Povysheva et al., 2006). Application of serotonin reduces the activity of GABAergic neurons (Puig et al., 2010) and reduces GABA release (Kruglikov and Bernardo, 2008). A recent study has extended these findings by proposing that the preferential activation of 5-HT_{1A}Rs on GABA cells results in disinhibition of pyramidal cells in cortex. Lladó-Pelfort et al. (2012) found that 8-OH-DPAT given intravenously reduced the discharge rate of mPFC fast-spiking GABAergic interneurons. This finding was partnered with the observation that administering 8-OH-DPAT intravenously produced an increase in the single unit firing rate of medial prefrontal cortex pyramidal neurons which could be reversed by WAY-100635 (Lladó-Pelfort et al., 2012). Studies using 8-OH-DPAT are cautioned because this drug possesses agonist activity for 5-HT_{1A}Rs and 5-HT₇Rs. In cortex, 5-HT₇Rs are present at low levels making it possible that they make some small contribution to the excitability changes observed with 8-OH-DPAT (Ruat et al., 1993; Hedlund and Sutcliffe, 2004). Together, studies from the literature indicate that activity of 5-HT_{1A}Rs either promote glutamatergic or reduce GABAergic transmission onto pyramidal cells in cortex.

This is the first study to investigate the role of serotonin on the expression of motor maps. We have shown that serotonin depletion diminishes normal motor map expression and results in forelimb movement deficits during skilled reaching. Based on sensitivity to WAY-100135 and 8-OH-DPAT, serotonin appears to exert an overall excitatory effect on motor cortex through 5-HT_{1A}Rs. This work raises the possibility that cortical excitabil-

ity may be altered in individuals with serotonin disorders or those receiving drug therapies that alter serotonin. 5-HT_{1A}Rs represent another therapeutic target for new treatments aimed at restoring cortical excitability in injury and disease states.

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