Behavioral/Cognitive

Increasing Central Serotonin with 5-hydroxytryptophan Disrupts the Inhibition of Social Gaze in Nonhuman Primates

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To competently navigate the world, individuals must flexibly balance distinct aspects of social gaze, orienting toward others and inhibiting orienting responses, depending on the context. These behaviors are often disrupted amongst patient populations treated with serotonergic drugs. However, those in the field lack a clear understanding of how the serotonergic system mediates social orienting and inhibiting behaviors. Here, we tested how increasing central concentrations of serotonin with the direct precursor 5-hydroxytryptophan (5-HTP) would modulate the ability of rhesus macaques (both sexes) to use eye movements to flexibly orient to, or inhibit orienting to, faces. Systemic administrations of 5-HTP effectively increased central serotonin levels and impaired flexible orientation and inhibition. Critically, 5-HTP selectively impaired the ability of monkeys to inhibit orienting to face images, whereas it similarly impaired orienting to face and control images. 5-HTP also caused monkeys to perseverate on their gaze responses, making them worse at flexibly switching between orienting and inhibiting behaviors. Furthermore, the effects of 5-HTP on performance correlated with a constriction of the pupil, an increased time to initiate trials, and an increased reaction time, suggesting that the disruptive effects of 5-HTP on social gaze behaviors are likely driven by a downregulation of arousal and motivational states. Together, these findings provide causal evidence for a modulatory relationship between 5-HTP and social gaze behaviors in nonhuman primates and offer translational insights for the role of the serotonergic system in social gaze.

Key words: **5-HTP; causal impairment; gaze inhibition; gaze orientation; nonhuman primates; serotonin**

Significance Statement

Behavioral changes arising from pharmacological agents that target serotonergic functions are complex and difficult to predict. Here, we examined the causal impacts of administering the direct precursor of serotonin, 5-HTP, on orienting and inhibiting social gaze in nonhuman primates. 5-HTP increased central concentrations of serotonin and selectively impaired the ability of monkeys to inhibit orienting to faces while similarly impairing the ability of monkeys to orient to face and control images. These behavioral gaze impairments were systematically associated with a downregulation of arousal and motivational states, indexed by pupil constriction, increased time to initiate trials, and increased reaction time. These findings provide a causal link between 5-HTP and social gaze behaviors in nonhuman primates and provide translational insights about serotonergic interventions.

Introduction

Competently navigating the world requires balancing the acts of orienting toward environmental stimuli and inhibiting orienting responses. This behavioral regulation demands coordination among the neural systems underlying motivation, inhibition, attention, and flexibility (Carver et al., 2008; Roberts et al., 2020). Among environmental stimuli, faces are particularly common and important for humans and nonhuman primates. Many species of primates, including humans, live in large, complex, societies and therefore orient to the faces of others to learn from conspecifics and to opportunistically gain information about

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fecundity and social status. However, many primate species must also inhibit orienting to faces to avoid accidentally aggressing to conspecifics and to avoid losing opportunities to gain nonsocial information about food sources and environmental risks (Weinberg-Wolf and Chang, 2019).

The serotonergic system seems to be critically involved in many aspects of cognition, which are central to this behavioral regulation. Among nonhuman primates, serotonergic function is implicated in social function and dominance status (Higley et al., 1996a,b; Westergaard et al., 1999; Fairbanks et al., 2001). Although the role of the serotonergic system in motivation has been well established among human and nonhuman primates (Carver et al., 2008; Roberts et al., 2020), the serotonergic system is also implicated in diverse aspects of behavioral inhibition (Roberts et al., 2020). Decreasing central serotonin disrupts impulse control not only in nonhuman primates (Clarke et al., 2004, 2005) and rodents (Harrison et al., 1997a, 1999) but also in humans (LeMarquand et al., 1999; Rubia et al., 2005; Crockett et al., 2010). Importantly, serotonin is also associated with flexibility; disrupting serotonergic function affects reversal learning and perseverance during choice tasks among many groups of mammals (Bailey et al., 2018; Lottem et al., 2018; Roberts et al., 2020). Finally, work has investigated the role of central serotonergic function and serotonergic genetics in nonhuman primate attention (Gibboni et al., 2009; Watson et al., 2015; Weinberg-Wolf and Chang, 2019), although this is an understudied field. Our lab has previously shown that acute delivery of the direct serotonin precursor 5-hydroxytroptohan (5-HTP) increases central concentrations of serotonin in macaques with concomitant changes in attention, particularly attention to faces of conspecifics (Weinberg-Wolf et al., 2018).

In humans, modulating serotonin function has been shown to alter cognitive biases, attention, and motivation, with these effects often being strongest within the social domain (Young, 1996; Riedel, 2004; Merens et al., 2007; Mendelsohn et al., 2009; Silber and Schmitt, 2010; Roberts et al., 2020). Substantial research has investigated more specifically how modulating serotonin function affects attentional biases to different classes of emotional stimuli among healthy and patient populations and suggests that decreasing central serotonin with acute tryptophan depletion causes negative attentional biases (Klaassen et al., 2002; Munafò et al., 2006; Fusar-Poli et al., 2007; Roiser et al., 2008; Robinson et al., 2010). Conversely, increasing circulating serotonin seems to modulate eye gaze patterns (Jonassen et al., 2015) and decrease negative attentional biases and negative emotional recognition (Luciana et al., 2001; Harmer et al., 2003, 2004, 2006; Murphy et al., 2006; Jonassen et al., 2015). These findings collectively support the idea that the serotonergic system is well positioned to affect flexible social orienting behaviors.

In clinical settings, disruptions in central serotonergic functions are associated with autism spectrum disorder (ASD) and Williams syndrome (WS; August and Realmuto, 1989; Williams et al., 2011; Barak and Feng, 2016; Muller et al., 2016; Fan et al., 2020; Lew et al., 2020). ASD and WS are also both associated with deficits in social orienting. Infants with ASD lack early social predispositions (Klin et al., 2002; Dawson et al., 2004), and these deficits continue into adulthood (Pelphrey et al., 2002; Kliemann et al., 2010). Evidence further suggests that the deliberate recognition of, and orienting toward, different emotional expressions are impaired in ASD individuals (Sigman et al., 1992; Bacon et al., 1998; Humphreys et al., 2007). On the other hand, individuals with WS, a rare genetic disorder associated with hypersociality, exhibit heightened social engagement, increased attention to faces, and uninhibited approach to strangers (Barak and Feng, 2016).

Indeed, serotonergic agents are broadly prescribed to individuals with social disorders such as ASD and WS (Williams et al., 2011; Muller et al., 2016) and also those suffering from numerous other psychiatric disorders including depression, anxiety disorders, and personality disorders (Abi-Dargham et al., 1997; Dell'Osso et al., 2010; Cowen and Browning, 2015; Healy, 2015). However, outcomes of serotonergic interventions for individuals with social, and other, disorders are variable and difficult to predict (Shaw et al., 2002; Turner et al., 2006; Chekroud et al., 2016). To better understand the effect of serotonergic interventions, it is important to conduct basic science research into the role of serotonin on aspects of cognitive functions that are impaired in these disorders in patients but also in healthy populations. To best conduct these studies, controlled, causal, experiments are required.

Causal manipulation studies of serotonergic function on behavior, especially repeated within-subject studies, are rare. They often require animal models, among which nonhuman primates are ideal for their similarities in behavior and anatomy. Despite growing evidence for the role of serotonin in cognition, particularly motivation, inhibition, and attention, it remains unclear how increasing central serotonin availability affects social gaze inhibition along with the flexible balancing of orienting and inhibiting gaze. Causal characterizations of such behavioral changes in nonhuman primate models, in which these behaviors are understudied, will help support basic science understandings of the serotonergic system and are critical for continued translational efforts. Here, we used a rhesus macaque model to examine how increasing central serotonin with 5-HTP modulates social gaze behaviors when monkeys were cued to either orient toward faces or inhibit this orienting response.

Materials and Methods

Animals. Three adult (two male and one female; monkeys H, T, E; age 7 years) rhesus monkeys (*Macaca mulatta*) served as subjects. Subjects weighed between 10 and 15 kg throughout the duration of the study. In a socially configured colony room with visual and auditory access to other conspecifics, subjects were either housed without a pair mate (n = two of three) or with a single pair mate (n = one of three), kept on a 12 h light/dark cycle, had access to food 24 h a day *ad libitum*, and had controlled access to fluid during testing. All procedures were reviewed and approved by the Yale University Institutional Animal Care and Use Committee.

Surgery. All subjects received a surgically implanted head-restraining prosthesis (Gray Matter Research) to allow accurate tracking of eye movements. At the time of surgery, anesthesia was induced with ketamine hydrochloride (10 mg/kg) and maintained with isoflurane (1.0–3.0%, to effect). During surgery, subjects received isotonic fluids via an intravenous drip, and aseptic procedures were used. Heart rate, respiration rate, blood pressure, expired CO_2, and body temperature were monitored throughout the procedure. After the head restraining device implantation was completed, the wound around the base was closed in anatomic layers. Subjects received a perioperative and postoperative treatment regimen consisting of 0.01 mg/kg buprenorphine every 12 h for 3 d, 0.1 mg/kg meloxicam once daily for 3 d, and 5 mg/kg baytril once daily for 10 d. Subjects were allowed 40+ d of recovery after the implant surgery before training began and were slowly acclimated to head restraint over a week of training.

Pharmacological methods. All pharmacological treatments were administered daily (consistently at either 12:30 or at 16:00, depending on the subject and whether data were collected from a given monkey as the first or second subject on a given day) and by intramuscular (i.m.) injection exactly 1 h before testing. Administered volume was consistently

between 3.0 and 5.0 ml depending on the weight of the subject. 5-HTP (Sigma-Aldrich) was suspended in sterile water and given at 20 mg/kg. Vehicle injections consisted of equal volumes of sterile saline. Each subject received a total of eight injections of saline and eight injections of 20 mg/kg 5-HTP on strictly alternating days. The dose of 5-HTP was selected on the basis of our previous work (Weinberg-Wolf et al., 2018) indicating that 20 mg/kg 5-HTP effectively increases the concentration of 5-HTP and serotonin in cisternal CSF. Previous studies in rodents and human subjects (Griebel, 1995; Turner et al., 2006) have indicated that 5-HTP doses <20 mg/kg do not reliably produce discernable behavioral effects, whereas doses >60 mg/kg can inadvertently increase circulating catecholamines by displacing them from storage granules, thereby temporarily enhancing postsynaptic catecholaminergic stimulation (Lichtensteiger et al., 1967).

Cisternal CSF sample collection and assays. To confirm whether an intramuscular injection of 5-HTP crossed the blood–brain barrier in rhesus macaques and to test whether injections indeed increased central levels of 5-HTP as well as serotonin, we sampled CSF from the cisterna magna from five rhesus monkeys (four male and 1 female; monkeys E, H, T, K, L; age 5–8 years, 5.5 ± 1.2) after they had received an intramuscular injection of saline or 20 mg/kg 5-HTP with a minimum of 2 weeks between each CSF collection date. We counterbalanced and randomized the subject order of CSF sampling between saline and 5-HTP. Each CSF draw occurred 1 h postinjection, the same time after injection that data collection on animals began in the current study.

Cisternal CSF was obtained with cervical punctures, which are preferred over lumbar punctures for accurately tracking concentrations of monoamines and monoamine metabolites in cortical and subcortical structures because of its greater proximity to the brain and its clearance from the spinal space (Anderson et al., 1987b, 2002, 2005). Punctures targeted the cisterna magna through the atlanto-occipital membrane. Approximately 1.5 ml of CSF was drawn using a 24–27 gauge needle. Monkeys were first anesthetized with ketamine (3 mg/kg, i.m.) and dexdomitor (0.075 mg/kg, i.m.), and anesthesia was reversed with antisedan (0.075 mg/kg, i.m.) once the animal was returned home. CSF was immediately labeled and frozen on dry ice before being transferred to a −80-degree Celsius freezer.

Samples with gross blood contamination (>0.1%), as indicated by pink coloration, were excluded before screening for hemoglobin. Limiting blood contamination to <0.1% was sufficient to ensure that analyses other than serotonin were not affected by blood. However, all CSF samples analyzed for serotonin were screened more rigorously for blood contamination by measuring hemoglobin using Multistix 8 SG reagent strips for urinalysis (Bayer), which can detect ~0.2 μg/ml of hemoglobin. As previously demonstrated, screening for hemoglobin and using only those samples with <10 ppm blood limited blood-derived serotonin in CSF to <10 pg/ml (Anderson et al., 2005). Neurochemical analyses levels of CSF 5-HTP and serotonin were determined using reverse-phase high-performance HPLC as previously described (Anderson et al., 1987a,b, 1990, 2002).

Samples with any detectable blood contamination were excluded from analysis; a total of two samples were excluded. The final 5-HTP CSF data therefore included samples collected after injection of saline and 20 mg/kg 5-HTP from four subjects with an additional subject contributing a sample at saline. The final serotonin CSF dataset was more restricted and includes saline data from four subjects and 20 mg/kg 5-HTP data from three subjects. Changes in CSF concentrations of 5-HTP and serotonin were each assessed using a two-tailed independent *t* test.

Experimental design. Monkeys performed the experimental task while sitting in a primate chair (Precision Engineering) in a testing room and used eye movements to interact with stimuli on an LCD monitor, positioned 36 cm away from the subject with a temporal resolution of 2 ms. MATLAB (MathWorks) with Psychtoolbox (Brainard, 1997) and EyelinkToolbox (Pelli, 1997) was used to display stimuli and process eye position data. Horizontal and vertical eye positions were sampled at 1,000 Hz using an infrared eye monitor camera system (EyeLink, SR Research). Monkeys initiated each trial by fixating on a white fixation square (7.3 × 7.3° visual) at the center of the screen for 150 ms. On successful fixation, a central instructional cue (7.3 × 7.3°) appeared at the center of the screen, and at the same time, a target image (9.8 × 9.8°) appeared in the right or left periphery of the screen at a 29.3° eccentricity. On 50% of pseudorandom trials (orienting trials), the central instructional cue was a red square, directing the subject to saccade to the peripheral target image to receive a juice reward (Fig. 1*A*). On the other 50% of pseudorandom trials (inhibition trials), the instructional cue was a blue square, directing subjects to inhibit orienting their gaze toward the target image to receive the reward (Fig. 1*A*). From the time of image onset, the subjects had 750 ms to orient or inhibit orienting to the image. Images disappeared as soon as the eyes of the subjects entered the image (either on correct orientating trials or incorrect inhibition trials) or after the 750 ms window on correct inhibition trials. A solenoid valve controlled the delivery of 0.5 ml of apple juice per each correct orienting or inhibiting response.

The peripheral visual images were unaltered conspecific face stimuli taken from a large library of static monkey face images described by Gothard et al. (2004). Stimulus monkeys displayed either threatening expression or affiliative lip smacks with a direct gaze. Subjects had never interacted with any of the monkeys depicted in these images. We divided the total number of images into four unique sets. Each set consisted of 24 conspecific faces per each of the two facial expression categories, resulting in 48 unique faces per set for a total of 192 images. Each image set also contained equal numbers (48 images per set) of luminance-matched scrambled faces (control images). We collected 2 d of data per each image set for each drug condition. To preclude any order effects, we counterbalanced the order in which we selected image sets while ensuring that subjects were never exposed to the same set of images during two sessions in a row. Within a single session of data collection, we also counterbalanced and randomized the order of image presentation to preclude any order effects.

The behavioral training took place in the following manner. Monkeys were first trained to perform orientation (O) and inhibition (I) trials separately. Then, they were trained to perform both trial types on a given day in alternating blocks of 200 trials. The trial duration of each block was progressively reduced until the two trial types were pseudorandomly interleaved without any block structure. Crucially, the behavioral testing on the effects of 5-HTP and saline on these trials commenced once monkeys reached a plateau in performance of each trial type based on 5 consecutive days. Initiated trials were defined as those in which the monkeys successfully held gaze fixation for 150 ms during the fixation period at the beginning of the trial and thus successfully progressed to view an instructional cue and target image. Upon breaking fixation during this period, the trial was aborted, no cue or image appeared, and the animal was not rewarded. Instead, they received a 1.5 s time out with a blank screen. In addition, we excluded initiated trials from the analysis that were associated with a pupil size during the fixation window that was more than 2 SDs away from the mean pupil size within drug condition, trial type, and image category. Using this criterion, we only excluded 3% of initiated trials.

Statistical analysis. All statistical analyses were conducted in MATLAB, and analysis code is available at Github. To compare saccade kinematics between 5-HTP and saline, we calculated the peak saccade velocity (degree/s) of orienting saccades (made during the 750 ms window aligned to the onset of the instructional cue and the visual image; Fig. 1*A*) and plotted them as a function of the peak saccade amplitude (degree) for each saccade during 5-HTP and saline sessions, separately for trials with faces and control images. To examine gaze fixation patterns of successfully performed trials, we constructed fixation density maps of the screen, spanning both the instructional cue and the target image locations, for all gaze fixations made from 300 to 750 ms (aligned to the onset of the instructional cue and the visual image) separately for 5-HTP and saline trials. We averaged how many gaze samples occurred in bounds of the stimulus, separately for the left and right target image and the instructional cue. We then tested for any differences in the number of gaze samples between 5-HTP and saline sessions, separately for orientation and inhibition trials, using Wilcoxon rank sum tests.

To further investigate the effect of 5-HTP on gaze behavior during inhibition trials, we calculated the percentage of inhibition trials in which monkeys remained fixated on the central cue. We also queried the

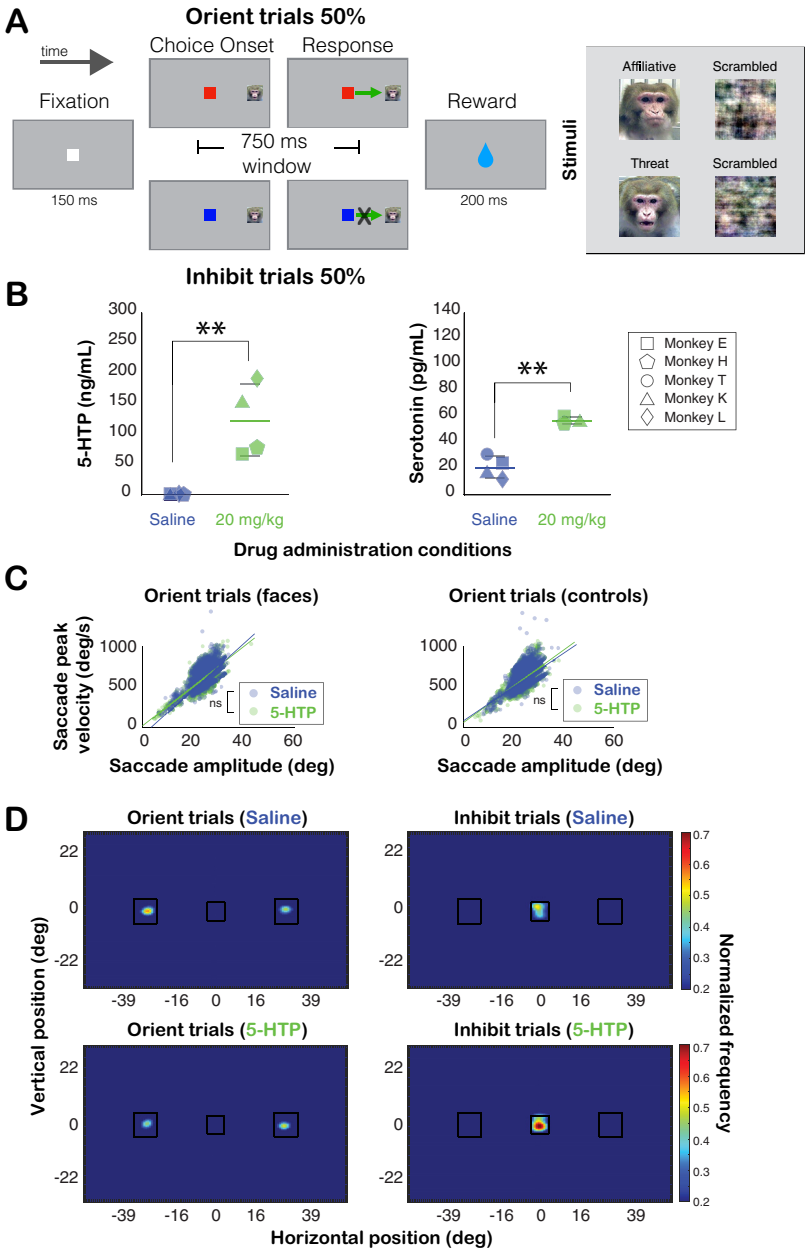

Figure 1. Behavioral task and CSF concentrations of 5-HTP and serotonin following 5-HTP and saline administrations. ***A***, Behavioral task sequence. Example face and luminance-matched scrambled control images are shown on the right. The target image appeared either at the right or left of the instructional cue (see above, Materials and Methods). ***B***, Left, CSF concentration of 5-HTP illustrating the central concentration of 5-HTP after intramuscular injection of saline (blue) or 20 mg/kg 5-HTP (green). Right, CSF concentration of serotonin illustrating the central concentration of serotonin after intramuscular injection of saline (blue) or 20 mg/kg 5-HTP (green). The average CSF concentration per condition is represented by a colored line, and shorter gray lines represent SE. Each shape represents data for individual monkeys. $^{**}p < 0.01$, *t* test. ***C***, Quantifications of saccade kinematics during successful orient trials to faces (left) and control images (right) during both 5-HTP (blue) and saline (green) sessions. ***D***, Fixation density heat maps (normalized fixation frequency) of correct orient and inhibit trials for 5-HTP and saline conditions. Black outlines represent the stimuli (central instructional cue and left or right target image).

effect of 5-HTP on the variance of gaze fixations during inhibition trials. Finally, we asked whether 5-HTP had an impact on the number of microsaccades monkeys made during inhibition trials. We identified microsaccades by first filtering *x* and *y* gaze position traces from each trial, aligned to the onset of the cue, using a 7 ms Gaussian kernel. We then computed the gradient of each trace to yield instantaneous velocity and identified candidate saccade intervals where either *x* or *y* speed was above 100°/s. We computed the start time of each candidate saccade interval, then removed start times from intervals longer than 60 ms in time or >30° in amplitude. The remaining intervals were taken to be microsaccades. Differences in fixating on the cue, the variance of gaze patterns, and the number of microsaccades were each assessed using an ANOVA model that specified monkey identity (monkey E, monkey H, monkey T), drug (saline vs 20 mg/kg 5-HTP), trial outcome (correct vs incorrect), image type (face vs scrambled), and image valence (threat vs appetitive) as fixed factors.

Our primary measure of interest was performance in the task. To directly examine whether 5-HTP modulated performance, we calculated the percentage of initiated trials that monkeys completed correctly. Percent correct was assessed using ANOVA models. The first model specified animal identity (monkey E, monkey H, monkey T), drug (saline vs 20 mg/kg 5-HTP), trial type (orienting vs inhibition), image type (face vs scrambled), and image valence (threat vs appetitive) as fixed factors. The second model used data that were normalized relative to saline as a percent change in performance because of 5-HTP to control for individual differences among monkeys and specified trial type (orienting vs inhibition), image type (face vs control), and image valence (threat vs appetitive) as fixed factors. Finally, the third model examined performance separately for orienting and inhibition trials and specified image type (face vs control) and image valence (threat vs appetitive). Direct *post hoc* comparisons were made with two-tailed independent *t* tests, and *p* values were corrected for multiple comparisons with Tukey's tests. To further investigate the stability of performance over the course of a given session, we plotted performance over time for orienting or inhibiting orienting to face and scrambled images during saline and 5-HTP sessions. Performance was calculated across 240 s bins with 20 s steps. Differences in performance between saline and 5-HTP sessions were assessed by conducting a Wilcoxon signed-rank test for each of these bins.

To query whether 5-HTP affected flexibility in monkeys in the current task, we quantified performance (as percent correct) as a function of the following four preceding contexts: (1) the previous trial was correct and the same type (O or I) as the current trial (same, O→O or I→I); (2) the previous trial was correct, but it was a different trial type from the current type (switch, O→I or I→O); (3) the previous two trials were both correct and both the same as the current trial (same, O→O→O or I→I→I); and finally (4) the previous two trials were both correct but were both a different trial type than the current trial type (switch, O→O→I or I→I→O). We then controlled for individual differences among monkeys by quantifying the percent change in performance because of 5-HTP relative to saline and assessed differences using an ANOVA model that specified current trial type (orienting vs inhibition), previous trial type (same vs switch), and number of preceding trials (one vs two) as fixed factors. We then examined performance separately for orientation and

inhibition trials with separate ANOVA models, specifying the previous trial type (same vs switch) and the number of preceding trials (one vs two) as fixed factors. Direct *post hoc* comparisons were made with two-tailed independent *t* tests, and *p* values were corrected for multiple comparisons with Tukey's tests.

To examine whether 5-HTP had an impact how long monkeys took to initiate each trial, we quantified the time between the end of the intertrial interval and the next successfully initiated trial (i.e., acquiring the central fixation and maintaining the fixation for 150 ms) during saline and 5-HTP sessions and compared them using a Wilcoxon rank sum test. To determine whether the time taken to initiate trials was related to performance during each session, we correlated the percent change of each session in the time to initiate trials because of 5-HTP relative to saline and the percent change in performance because of 5-HTP relative to saline. For analyzing pupil size, we quantified the size of the pupil during the fixation period of initiated trials and compared pupil size between 5-HTP and saline sessions using a Wilcoxon rank sum test. We determined the relationship between pupil size and performance by correlating the percent change of each session in pupil size because of 5-HTP with the percent change in performance because of 5-HTP, both relative to saline. To examine the relationship between reaction time and performance, we first quantified reaction time as the time from cue/image onset to when animals began saccades to target images, using a 20°/s velocity criterion for detecting the saccade onset (i.e., reaction time). We assessed differences in reaction time during correct orienting trials using an ANOVA specifying animal identity (monkey E, monkey H, monkey T), drug (saline vs 20 mg/kg 5-HTP), image type (face vs scrambled), and image valence (threat vs appetitive) as fixed factors. We then correlated the percent change of each session in reaction time with the percent change in performance during orienting trials, both because of 5-HTP relative to saline. Direct *post hoc* comparisons were made with two-tailed independent *t* tests, and *p* values were corrected for multiple comparisons with Tukey's tests. Correlations were reported by calculating a Pearson's linear correlation coefficient.

Data availability. The data used in the article are available and downloadable from GitHub at https://github.com/changlabneuro/social-inhibition-5htp.

Results

Three rhesus macaques performed a task designed to test their ability to flexibly orient, or inhibit orienting, toward peripheral target images by using a colored instructional cue that appeared at the same time as the target image (Fig. 1*A*). Peripheral target images were unfamiliar conspecific faces or luminance-matched scrambled faces as controls. We tested the causal role of serotonin in orienting and inhibiting gaze responses by increasing central concentration of serotonin with the direct precursor, 5-HTP. Our main behavioral measure of interest was the percentage of trials correctly completed for both orienting and inhibition trials. We also investigated how the preceding behavioral context affected orienting and inhibition performance. Finally, we examined the effect of 5-HTP on pupil size, how long monkeys took to initiate trials, and reaction time to relate these measures to changes in performance because of 5-HTP.

5-HTP increases central serotonin and constricts the pupil

We have previously demonstrated that 5-HTP increases concentrations of both 5-HTP and serotonin in cervical CSF (Weinberg-Wolf et al., 2018). In all monkeys that participated in the CSF study, including the three monkeys we collected behavioral data from during the behavioral current study, CSF 5-HTP concentrations were higher after receiving 20 mg/kg 5-HTP compared with saline ($t_{(7)} = -4.68$, $p < 0.01$, $d = -1.65$, *t* test; Fig. 1*B*). Additionally, in all these monkeys, 5-HTP administration also increased central serotonin ($t_{(5)} = -6.91$, $p < 0.01$, $d = -1.78$, *t* test; Fig. 1*B*). Importantly, central concentrations of 5-HTP and serotonin were strongly correlated with one another, indicating that increases in serotonin are proportional to increases in 5-HTP ($r = 0.83$, $p = 0.02$, Pearson's correlation). Because cervical CSF taps are invasive procedures, we previously established a biomarker of the impact of 5-HTP on central serotonin; 5-HTP dose dependently decreases the size of the pupil (Weinberg-Wolf et al., 2018). We thus quantified the size of the pupil, following 5-HTP and saline administrations, during the 150 ms fixation period where a luminance-controlled white fixation square appeared alone on the screen and when the eyes of the animal were steady as they fixated. Monkeys exhibited a significantly more constricted pupil during 5-HTP sessions than saline sessions ($z = 5.25$, $p < 0.001$, Wilcoxon signed rank, $r = 0.76$; more later), and the magnitude of this constriction ($-26.09 \pm 2.07\%$, mean ± SEM) was consistent with our previous work ($-20.53 \pm 4.15\%$, mean ± SEM, $t_{(41)} = -1.32$, $p = 0.19$, $d = 0.45$, *t* test; Weinberg-Wolf et al., 2018). These cisternal CSF and pupil results support the notion that 5-HTP administrations effectively cross the blood–brain barrier to causally influence central serotoninergic function and the CNS.

Indifferent saccadic and fixation behaviors between 5-HTP and saline

To ensure that 5-HTP did not merely cause atypical gaze behaviors to influence performance, we compared saccade kinematics and gaze fixations during the task The relationship between peak saccade velocity and amplitude did not differ between 5-HTP and saline sessions when monkeys oriented to faces ($p = 0.94$, permutation test) or to control images ($p = 0.57$, permutation test; Fig. 1*C*). We also compared gaze fixation patterns on target image locations during successfully completed orient trials, as well as gaze fixations on any parts of the monitor screen during correctly completed inhibit trials, between 5-HTP and saline sessions. Fixation patterns during orient trials were not different between 5-HTP and saline (right and left visual image locations, both $z < 1.91$, $p > 0.06$, $r < 0.28$, Wilcoxon rank sum; Fig. 1*D*). Moreover, monkeys largely remained fixated on the central cue during successful inhibit trials (despite the fact that the task did not require them to do so). These fixation patterns did not differ across 5-HTP and saline sessions (saline sessions, $52.6 \pm 2.6\%$; 5-HTP sessions, $52.0 \pm 3.5\%$; $z = 0.01$, $p = 0.99$, $r = 0.003$, Wilcoxon rank sum; Fig. 1*D*) or across trials with social and nonsocial images ($F_{(1,184)} = 0.25$, $p = 0.60$, $e2 < 0.01$, ANOVA), nor those with threatening and appetitive images ($F_{(1,184)} = 0.02$, $p = 0.89$, $e2 < 0.001$, ANOVA; Fig. 1*D*). In addition, the variance in gaze fixations during inhibit trials were also similar across 5-HTP and saline sessions (saline sessions, 90.6 ± 9.5 px; 5-HTP sessions, 94.7 ± 8.8 px; $F_{(1,336)} = 2.17$, $p = 0.14$, $e2 < 0.001$, ANOVA), and between trials using social and nonsocial images ($F_{(1,336)} = 0.43$, $p = 0.51$, $e2 < 0.001$, ANOVA), as well as those trials with threatening and appetitive images ($F_{(1,336)} = 0.003$, $p = 0.96$, $e2 < 0.001$, ANOVA). Finally, animals made very few microsaccades during inhibition trials (correct trials, 2.0 ± 0.10; incorrect trials, 1.46 ± 0.07), and the frequency of microsaccades did not differ between 5-HTP and saline sessions (saline sessions, 1.83 ± 0.11; 5-HTP sessions, 1.82 ± 0.14; $F_{(1,336)} = 0.01$, $p = 0.91$, $e2 < 0.001$, ANOVA), between trials with social and nonsocial images ($F_{(1,336)} = 0.06$, $p = 0.81$, $e2 < 0.001$, ANOVA), nor those trials with threatening and appetitive images ($F_{(1,336)} = 0.28$, $p = 0.6$, $e2 < 0.001$,

ANOVA). Therefore, we did not find any evidence that 5-HTP affected low-level gaze behaviors.

5-HTP impairs orienting and inhibition performance

Our primary question was whether increasing central concentrations of serotonin using 5-HTP would affect the ability of monkeys to orient or inhibit orienting toward faces. We first assessed performance by quantifying the percentage of trials completed correctly over the course of a saline or 5-HTP session. We observed strong impairments in performance following 5-HTP (78.3 ± 1.7%, mean ± SEM) compared with saline (85.9 ± 1.1%; $F_{(1,336)} = 75.34$, $p < 0.001$, $e2 = 0.06$, ANOVA; Fig. 2*A*,*B*). We next queried how 5-HTP affected orienting and inhibition performance. Monkeys performed orienting trials at a near-ceiling performance after receiving saline (98.1 ± 0.2%), whereas they displayed orienting impairments after 5-HTP (90.8 ± 1.8%; $p < 0.001$, Tukey's test; Fig. 2*A*,*B*). After normalizing performance to saline, we found that 5-HTP impaired orienting performance similarly on trials with control images (raw 5-HTP, 88.7 ± 2.8%; raw saline, 98.3 ± 0.3%; −5.3 ± 1.6% change from saline) and those with face stimuli (raw 5-HTP, 92.8 ± 2.2%; raw saline, 97.9 ± 0.4%; −9.6 ± 2.0% change from saline; $F_{(1,92)} = 2.87$, $p = 0.09$, $e2 = 0.06$, ANOVA; Fig. 2*C*). We also asked whether orienting performance differed according to the expressions conveyed by face images, threatening versus affiliative. However, monkeys oriented to threatening and affiliative faces at similar rates ($F_{(1,184)} = 0.11$, $p = 0.74$, $e2 < 0.001$, ANOVA), and 5-HTP impaired performance during appetitive and threatening trials similarly ($F_{(1,92)} = 0.001$, $p = 0.97$, $e2 < 0.001$, ANOVA). Although this negative finding may seem surprising at first, it was likely because of the fact that target images in this task disappeared as soon as the monkeys gaze first entered the area, leaving no time for the monkeys to foveate on or scan them.

We next investigated performance on inhibition trials. Monkeys were worse at performing inhibition trials (74.4 ± 1.5%, mean ± SEM) compared with orienting trials (98.1 ± 0.2%; $F_{(1,184)} = 24.99$ $p < 0.001$, $e2 = 0.58$, ANOVA) during saline sessions (Fig. 2*A*,*B*), indicating that it was difficult for monkeys to inhibit looking at a stimulus in their periphery, and 5-HTP further decreased this performance (66.8 ± 1.5%, $p < 0.001$, Tukey's test; Fig. 2*A*,*B*). After normalizing performance to saline, we found that 5-HTP impaired inhibition performance more on trials with face images (raw 5-HTP; 66.8 ± 2.2%, mean ± SEM; raw saline: 74.7 ± 1.9%; −14.72 ± 1.9% change from saline) compared with those with control images (raw 5-HTP, 69.8 ± 1.9%; raw saline, 74.2 ± 2.3%; −5.36 ± 1.9% change from saline; $F_{(1,92)} = 12.16$, $p < 0.0001$, $e2 = 0.09$, ANOVA; Fig. 2*C*). We again observed no differences in inhibition performance between trials with threatening compared with appetitive faces ($F_{(1,184)} = 0.22$, $p = 0.64$, $e2 = 0.001$, ANOVA), and 5-HTP impaired performance during appetitive and threatening trials similarly ($F_{(1,92)} = 0.38$, $p = 0.54$, $e2 < 0.001$, ANOVA). Overall, these measures suggest that increasing central serotonin availability with 5-HTP impairs orienting and inhibiting gaze responses, but that faces, perhaps because of their inherent saliency, caused an especial impairment in the ability to inhibit orienting gaze toward them (Fig. 2*C*).

5-HTP reduces flexibility in orienting and inhibiting gaze responses

Work across multiple species has investigated the role of serotonin in inhibiting responses and flexibly changing response (Roberts et al., 2020). In the current task, monkeys were trained

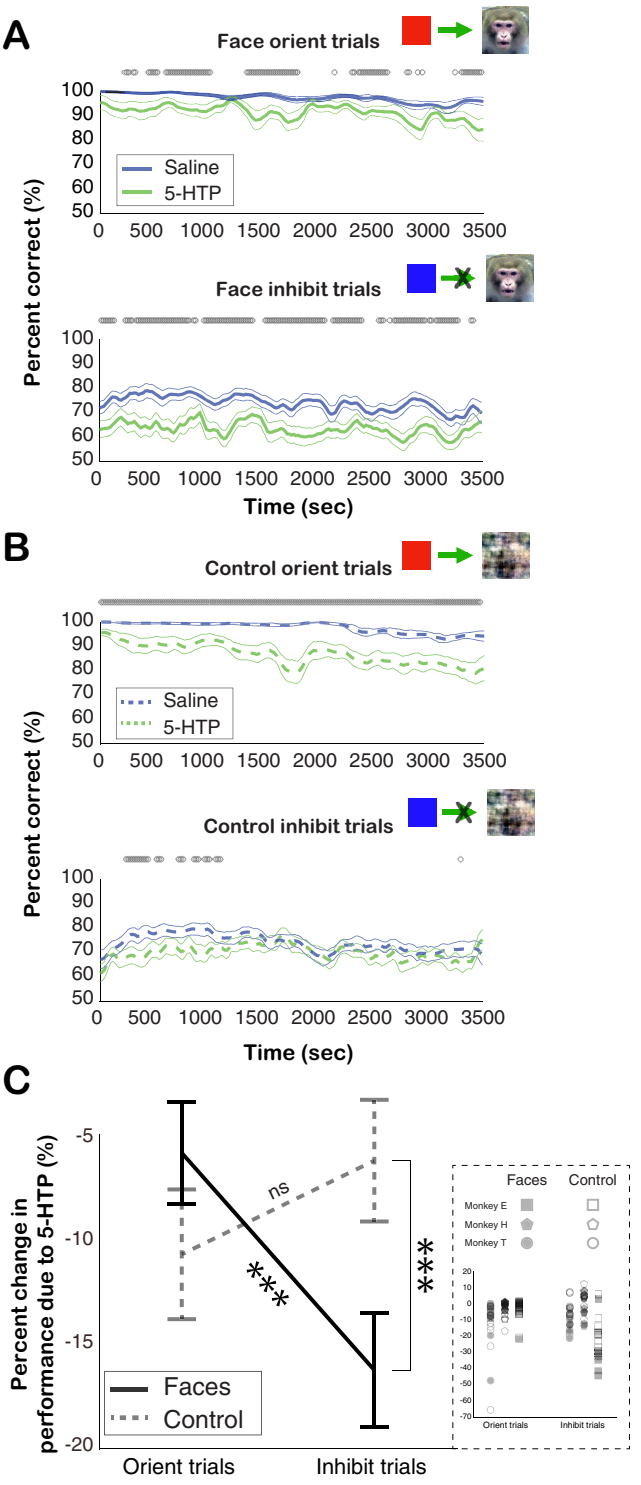

Figure 2. 5-HTP disrupts orienting and inhibition performance. ***A***, Average performance over the course of a session during orienting trials (top) and inhibition trials (bottom) for face images. ***B***, Average performance over the course of a session during orienting trials (top) and inhibition trials (bottom) for scrambled control images. Performance during saline sessions is shown in blue, whereas 5-HTP session is shown in green (mean ± SEM; ***A***, ***B***). The gray circles above each time series represent significant differences between 5-HTP and saline at each time point ($p < 0.05$, Wilcoxon signed rank; ***A***, ***B***). ***C***, The percent change in performance because of 5-HTP relative to saline (mean ± SEM), illustrating that 5-HTP selectively impaired performance on inhibition trials with face images (solid lines) but not those with control images (dashed line). ***$p < 0.001$; n.s., not significant, ANOVA. Inset, Each shape represents data from individual monkeys; filled shapes depict performance on trials with face images, and open shapes depict performance on trials with control images.

to use a central cue to flexibly switch between two responses, orienting or inhibiting orienting. We tested flexibility in this context by considering the perseverating effect of preceding trials (Durston et al., 2002). To this end, we compared performance on trials where the previous trials (one or two) had been correct in a row as well as in which the response type (i.e., orient or inhibit) was either the same as, or a switch from, the current trial type in four distinct conditions (see above, Materials and Methods).

After normalizing performance to saline, we found that 5-HTP disrupted performance more when the preceding trials were a switch from the current type (O→I, I→O, O→O→I, and I→I→O) compared with when they were the same (O→O, I→I, O→O→O, and I→I→I; $F_{(1,184)} = 5.73$, $p = 0.02$, $e2 = 0.03$, ANOVA). Notably, we found that 5-HTP worsened performance on inhibition trials that were preceded by correct orientation trials (O→I and O→O→I vs I→I and I→I→I; $F_{(1,92)} = 4.96$, $p = 0.03$) but only when there had been two (O→O→I vs I→I→I, $p = 0.03$, Tukey's test; Fig. 3) and not one (O→I vs I→I, $p = 0.99$, Tukey's test) preceding trial. By contrast, 5-HTP did not alter orienting performance according to the previous type (I→O and O→O→I vs O→O and O→O→O; $F_{(1,92)} = 1.11$, $p = 0.30$, $e2 = 0.008$, ANOVA) or previous trial sequence length (I→O and O→O vs O→O→I and O→O→O; $F_{(1,92)} = 0.73$, $p = 0.39$, $e2 = 0.0008$, ANOVA; Fig. 3). These findings suggest that 5-HTP increased the difficulty in inhibiting prepotent responses or, perhaps, that serotonin decreased the ability to flexibly switch between actions by increasing perseverance.

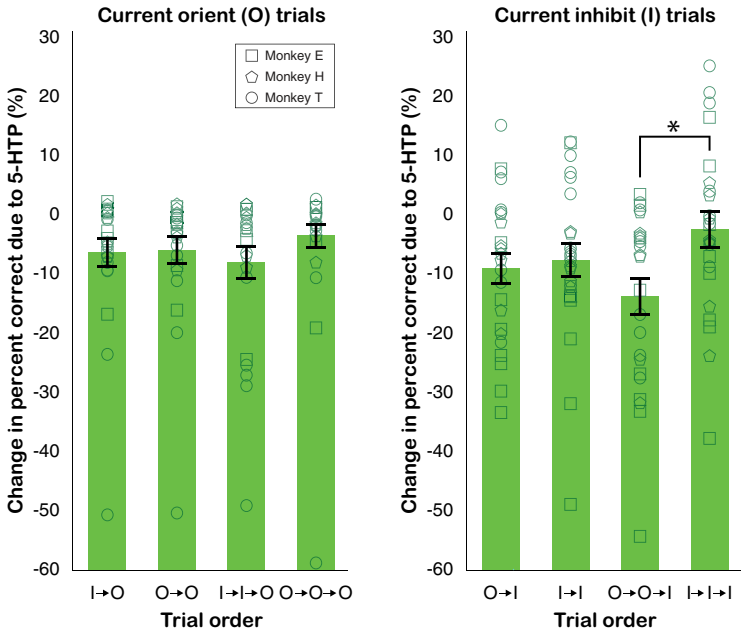

Figure 3. 5-HTP reduces flexibility in orienting and inhibiting gaze responses in monkeys. 5-HTP impaired performance when the preceding trials were a switch from the current trial type, but not when they were the same trial type (error bars indicate mean ± SEM); *$p < 0.05$, Tukey's test. Trial order is shown as a sequence of response types (O or I). For example, I→O indicates the current orientation trial followed a successfully completed inhibition trial in the previous trial. Similarly, O→O→I indicates the current inhibition trial followed a sequence of two successfully completed orientation trials. Each shape represents data from individual monkeys.

Decreases in task motivation and arousal by 5-HTP are related to performance impairments

Psychopathologies for which serotonergic interventions are relied on, especially depression, are commonly associated with decreased motivation and anhedonia (Grahek et al., 2019). For this reason, we were curious whether 5-HTP altered monkeys' task engagement in the present task. To this end, we calculated the time it took for monkeys to successfully initiate a trial (see above, Materials and Methods) based on the reasoning that under a more engaged state, monkeys ought to initiate trials more quickly compared with under a less engaged state. Monkeys took longer to initiate trials during 5-HTP sessions (5-HTP: 1800 ± 356 ms, mean ± SEM; saline: 583 ± 50 ms, $z = 1.97$, $p = 0.049$, $r = 0.28$, Wilcoxon rank sum; Fig. 4A, left). Crucially, after normalizing the number of initiated trials and performance of each session to saline, we found that the magnitude of the effect of 5-HTP on task engagement and performance were strongly, negatively correlated with one another ($r = -0.80$, $p < 0.001$; Fig. 4A, right). Thus, 5-HTP concurrently increased the intertrial initiation time and decreased performance on those trials.

Pupil size is also related to arousal state, motivation, and engagement (Zekveld et al., 2014; Peysakhovich et al., 2015; van der Wel and van Steenbergen, 2018; Shechter and Share, 2021). Therefore, we were also interested in examining whether the effects of 5-HTP on performance were related to 5-HTP-induced pupil constriction. To answer this question, we calculated the percent change in pupil size during the pretrial fixation period (controlling for luminance and eye position) because of 5-HTP for each session and correlated it with the percent change in performance because of 5-HTP. We first quantified the size of the pupil following 5-HTP and saline administrations during the 150 ms fixation period where a luminance-controlled white square appeared alone on the screen and when the eyes of the monkeys were steady as they fixated. As mentioned earlier, 5-HTP constricted the pupil compared with saline by 26% ($z = 5.25$, $p < 0.0001$, $r = 0.76$, Wilcoxon signed rank; Fig. 4B, left). Notably, we observed a positive correlation between these two variables ($r = 0.45$, $p = 0.028$, Pearson's correlation; Fig. 4B, right), indicating that the more 5-HTP constricted the pupil during a session, the more it also impaired performance. Given that 5-HTP may index arousal state, this finding further suggests that 5-HTP-induced impairments in task performance were linked to serotonin-mediated changes to arousal state.

5-HTP concomitantly increases reaction time and impairs gaze orienting responses

Reaction time is commonly used to study attention (Prinzmetal et al., 2005) and motivation (Mir et al., 2011). We were curious about whether increasing central concentrations of serotonin with 5-HTP would affect the reaction time of gaze orienting responses in the current task. Reaction time was longer during 5-HTP sessions (205 ± 3 ms, mean ± SEM) compared with saline sessions (188 ± 2 ms; $F_{(1,92)} = 33.61$, $p < 0.001$, $e2 = 0.27$, ANOVA; Fig. 4C, left). Reaction time did not, however, vary between trials with face and control images ($F_{(1,92)} = 1.11$, $p = 0.29$, $e2 = 0.009$, ANOVA) or threatening and appetitive faces ($F_{(1,92)} = 0.08$, $p = 0.78$, $e2 < 0.001$, ANOVA). We next queried the potential relationship between reaction time and performance during orientation trials by correlating the percent change in reaction time and the percent change in performance because of 5-HTP. Changes in reaction time were negatively correlated

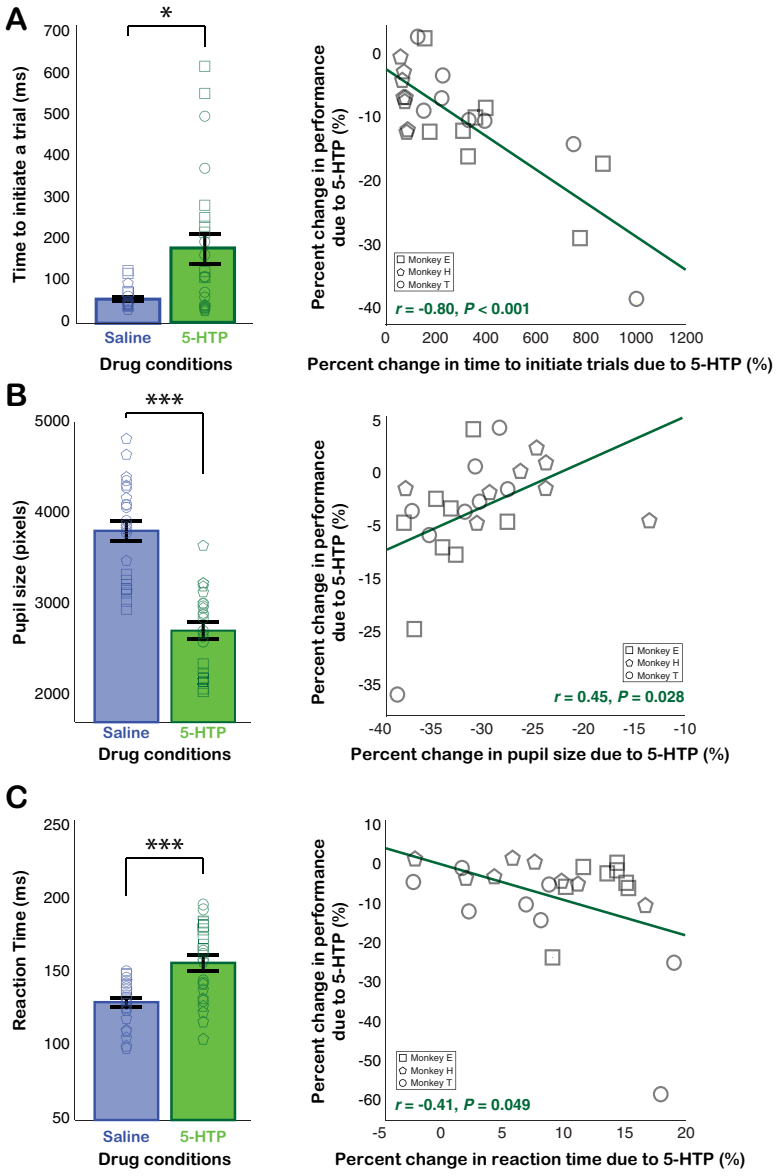

Figure 4. 5-HTP increases intertrial initiation time, constricts the pupil, and increases reaction time with concomitant changes in performance. ***A***, Left, 5-HTP increased the amount of time monkeys took before initiating a trial (mean ± SEM; individual session data are overlaid with data from each monkey using different shapes). Right, The percent changes in the intertrial initiation time were correlated with the percent changes in performance because of 5-HTP. ***B***, Left, 5-HTP constricted the pupil (same format as in ***A***, left). Right, The percent changes in pupil constriction were correlated with the percent changes in performance because of 5-HTP. ***C***, Left, 5-HTP increased reaction time (same format as in ***A***, left). Right, The percent changes in reaction time were correlated with the percent changes in orientation trial performance because of 5-HTP. Saline sessions are shown in blue, whereas 5-HTP sessions are shown in green. Each shape represents data from individual monkeys. $*p < 0.05$, $***p < 0.001$, *t* test. The green lines in the scatter plots (***A–C***) illustrate linear regression fits.

with changes in performance ($r = -0.41$, $p = 0.049$, Pearson's correlation; Fig. 4C, right) so that the more 5-HTP increased reaction time in a given session, the more it also impaired orientation performance. This finding suggests that changes in reaction time may index a state change in attention, engagement, or motivation, which is associated with 5-HTP impairments to orienting gaze responses.

Discussion

Navigating one's social environment requires individuals to flexibly orient to others, or inhibit orienting to them, depending on the context. Although there are many benefits associated with collecting social information, there are also costs. Monitoring others not only causes animals to miss out on gaining food sources, but it also leaves them less time to monitor the environment for predators and environmental risks. There are also social risks associated with social gaze; direct eye contact can be considered a threat to rhesus macaques (Maestripieri, 1997), and macaques avoid the escalated aggression that can follow inappropriate social threats (Higley et al., 1996b). Social gaze behaviors are therefore critical to competent social behavior, which is hypothesized to be associated with serotonergic function (Weinberg-Wolf and Chang, 2019). In the current study, we tested how acute administrations of 5-HTP would alter social orienting responses of macaques using gaze. 5-HTP selectively impaired social inhibition performance during trials with face images, whereas it impaired performance on all orienting trials similarly. Although our scrambled images of faces were matched for low-level characteristics like luminance, faces are still more salient and attentionally capturing (Shepherd et al., 2010). Therefore, faces might have captured attention more effectively and interacted with the effects of 5-HTP to disrupt inhibition performance more strongly.

Alternating between orienting and inhibiting responses requires flexibility. 5-HTP decreased performance on inhibition trials that were preceded by orienting trials, suggesting that 5-HTP induced monkeys to perseverate and become less flexible in switching between orienting and inhibiting gaze responses. Flexibility has traditionally been studied using reversal learning tasks, and individual differences in flexibility have been related to serotonergic function (Barlow et al., 2015), serotonergic neurons (Matias et al., 2017), and differences in serotonergic-related genetics (Izquierdo et al., 2007). In addition, causally depleting serotonin, particularly in the orbitofrontal cortex, impairs reversal learning (Clarke et al., 2004, 2005; Walker et al., 2009) and increases stimulus stickiness (Rygula et al., 2015). Interestingly, a study has reported that oral 5-HTP impairs decision-making in an Iowa Gambling task and decreased the likelihood that subjects would switch choices between decks in the task (Gendle and Golding, 2010). Although these effects do not seem to have been because of increased perseverance, they do indicate a reduction in flexible decision-making (Gendle and Golding, 2010). The serotonergic system is extremely complex with 14 serotonin receptor subtypes, each with differing functions (Roberts et al., 2020). For example, studies have shown that antagonizing the 5-HT2A receptor impairs performance in reversal learning tasks, whereas antagonizing the 5-HT2C receptor enhances reversal performance (Boulougouris et al., 2008; Furr et al., 2012; Nilsson et al.,

2012). Additionally, serotonergic drugs can also have an impact on behavioral outcomes in a categorially different manner according to individual differences and differences in drug doses (Weinberg-Wolf and Chang, 2019). Indeed, it has been shown that although a small, acute dose of the selective serotonin reuptake inhibitor (SSRI) citalopram impairs reversal learning, a high, acute dose had the opposite effect (Bari et al., 2010). Therefore, the impairment we observed in the current study, rather than improvement, in performance and flexibility is not wholly unexpected.

Disruptions to the serotonergic system have been casually linked to action impulsivity, especially through early responding in five-choice serial reaction time tasks and increases in firing rates of dorsal raphe nucleus neurons when rodents must wait for delayed rewards (Harrison et al., 1997a,b; Winstanley et al., 2005). Contrary to what one might predict from these results, increasing central serotonin with 5-HTP impaired inhibition performance in our task. It is worthwhile to note that our task was not designed to measure action impulsivity. Although inhibition trials were more difficult for monkeys, the current task did not build a prepotent response as observed in the traditional go/no-go or stop-signal reaction time paradigms because orienting trials and inhibition trials occurred randomly and at equal probability, and the target image appeared equally on the left and right side of the screen. Instead, our task required monkeys to use an instructional cue to select the appropriate gaze response.

The observed effects are likely mediated by the effects of serotonin on arousal state as indexed by pupil size. Mental strain and focus increase the size of the pupil (Zekveld et al., 2014; Peysakhovich et al., 2015; van der Wel and van Steenbergen, 2018; Shechter and Share, 2021), whereas sleepiness and fatigue decreases pupil size (Hopstaken et al., 2015). Our lab has established that 5-HTP dose-dependently constricts the size of the pupil (Weinberg-Wolf et al., 2018), an effect we have replicated in the current study. This biomarker indicates that 5-HTP has a consistent effect on the CNS. More specifically, we found that the change in pupil size was correlated with a change in performance so that the more 5-HTP constricted the pupil during a session, the more it also impaired performance. In contrast to our results, SSRIs have been shown to dilate the pupil (McDougal and Gamlin, 2011), as does phasic activation of serotonergic neurons (Cazettes et al., 2021). Furthermore, serotonin syndrome, associated with pathologically high levels of serotonin, is also associated with pupil dilation (Alusik et al., 2014). However, early causal studies conducted directly with serotonin and 5-HTP reported causal pupil constriction (Reid and Rand, 1952; Page, 1954; Wada and McGeer, 1966; Rapport, 1997), suggesting potentially different downstream effects from elevating serotonin availability using 5-HTP versus through SSRIs. Our 5-HTP manipulations could be upregulating the parasympathetic nervous system, downregulating the sympathetic nervous system, or a combination of the two. This effect on arousal may be critically underlying our observed changes in performance.

Although 5-HTP caused an amplified impairment to inhibition trials with face images, 5-HTP also overall impaired performance in the task. It is therefore likely that the observed effects of 5-HTP are, at least in part, because of general changes to motivational state. Overall, the percent change in the time monkeys took to initiate a new trial was negatively correlated with the percent change in performance so that the more 5-HTP increased the time to initiate a trial, the more it also impaired performance. This metric likely indexes motivational state and task engagement. It is worthwhile to note that certain conditions for testing flexibility can be confounded with task difficulty, and this change in task difficulty could in part drive the reduction in motivation to complete a trial correctly. Reaction time has also been used to estimate engagement (Mir et al., 2011). Here, the logic dictates that the more engaged animals are in a task, or the more motivated they are to view an image, the faster their reaction time would be. 5-HTP caused reaction times to slow with concomitant changes in performance, further supporting the proposed role of 5-HTP in affecting motivational state. It should be noted, however, that an increase in reaction time alone may not necessarily signify a decrease in engagement. For example, a previous study in humans found that an acute dose of the SSRI citalopram increased participant's response time and led to more careful moral decision-making (Crockett and Cools, 2015). Still, an earlier study investigated the effects of 30 mg/kg 5-HTP on a bar-pressing task in monkeys and found that 5-HTP transiently abolished bar pressing with concomitated pupil constriction, sleepiness, and decreased interest in favored foods (Wada and McGeer, 1966). We thus hypothesize that the effects of 5-HTP on overall performance and the ability to flexibly switch between trial types are primarily driven by a downregulation of arousal and motivational states, resulting in a constricted pupil, decreased number of trials initiated, slowing of reaction time, and impairment in performance. We also note that the difficulty associated with switching trials in a certain trial sequence (i.e., O→O→I; Fig. 3) may decrease motivation in animals and result in poorer performance. Understanding precisely how the serotonin system mediates the motivational state and the difficulty associated with flexible behavior requires further investigation.

Deficits in motivation are common in mood disorders (Grahek et al., 2019). Although SSRIs are used to treat depression, they are also associated with indifference and decreased sexual motivation (Roberts et al., 2020). In addition, increased brain serotonin has been associated with decreased motivation to seek rewards (Roberts et al., 2020). However, SSRIs also sometimes increase motivation (Meyniel et al., 2016), although these effects could be because of inadvertent activation of the dopaminergic system (Subhan et al., 2000). Furthermore, among those at risk of depression, decreasing central serotonergic function with acute tryptophan depletion can decrease motivation (Roiser et al., 2006). The time line of serotonergic interventions also seems to be critical. SSRIs often require chronic administration and can even cause a worsening of symptoms in patients at first (Duman et al., 2016; Roberts et al., 2020). In support of time-dependent effects of serotonin, optogenetically driving serotonergic cells was found to decrease acute, spontaneous locomotion in mice, but repeated stimulation was found to increase spontaneous locomotion in the aggregate (Correia et al., 2017). Given the complex autoreceptors associated with the serotonergic system, acute and chronic manipulations may result in variable outcomes.

Overall, our findings provide causal evidence that acutely increasing central serotonin with the direct precursor 5-HTP impairs the ability to flexibly orient or inhibit orienting to faces, likely by affecting the motivational and arousal systems. Future work replicating these findings could further clarify the mechanism underlying these effects by directly testing the effects of 5-HTP on reversal learning, motivation, social attention, and engagement. In general, 5-HTP is highly understudied, and the field would benefit from dissecting exactly how intramuscular injection of 5-HTP affects serotonergic receptor binding

throughout the brain. Continued efforts toward better understanding the causal effects of 5-HTP on behaviors, circuits, and receptors would clarify the relationship between the serotonergic system and complex behaviors, and could therefore help improve matching treatment outcomes with patients.

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