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# Testosterone biases the amygdala toward social threat approach

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**Testosterone enhances amygdala reactions to social threat, but it remains unclear whether this neuroendocrine mechanism is relevant for understanding its dominance-enhancing properties; namely, whether testosterone biases the human amygdala toward threat approach. This pharmacological functional magnetic-resonance imaging study shows that testosterone administration increases amygdala responses in healthy women during threat approach and decreases it during threat avoidance. These findings support and extend motivational salience models by offering a neuroendocrine mechanism of motivation-specific amygdala tuning.**

## INTRODUCTION

Testosterone lies at the core of social interactions by facilitating social approach and dominance-seeking behavior across species (*1*, *2*). In humans, testosterone administration leads to enhanced amygdala reactivity to angry faces. This effect has traditionally been interpreted as reflecting increased vigilance to social threat, and potentially resulting in increased aggression (*3*–*6*). However, threat vigilance can prime divergent motivational reactions, namely, threat approach, but also threat avoidance. Threat approach has been theoretically and empirically linked to social dominance, whereas threat avoidance is a clear sign of social submissiveness (*1*, *7*–*9*). In line with this, recent motivational salience theories predict that increased amygdala activity does not reflect emotional salience but is rather a function of motivational salience (*10*, *11*).

Therefore, to facilitate adaptive responding, testosterone should modulate amygdala activity on the basis of the motivational context—and not the emotional context per se. More precisely, approach-enhancing effects of testosterone during social challenges (*12*–*14*) should be supported by enhanced amygdala reactivity during motivational approach and not during avoidance. We tested this hypothesis using a double-blind, randomized, placebo-controlled testosterone administration study, combining functional magnetic resonance imaging (fMRI) with a well-established social approach-avoidance (AA) task in which angry and happy faces have to be approached or avoided by pulling or pushing a joystick, respectively (*15*, *16*).

During the AA task, a discrepancy between task instructions and automatic action tendencies of avoiding angry and approaching happy faces leads to increased activation of the anterior prefrontal cortex (aPFC), which coordinates the contribution of several brain regions, including the amygdala (*15*–*17*). In particular, the aPFC down-regulates amygdala responses when the internally driven emotional response tendencies need to be controlled according to externally driven task instructions.

Here, we tested for motivation-specific effects of testosterone on amygdala function. A single dose of 0.5 mg of testosterone or placebo was administered to 54 healthy females 4 hours before acquiring fMRI measurements. This procedure has been extensively validated in previous social affective neuroscience research (*4*, *12*, *18*, *19*). Here, salivary testosterone levels were increased when functional data were obtained (see Table 1).

## RESULTS

As expected on the basis of motivational salience accounts (*10*, *11*), motivation-specific effects in the amygdala were modulated by testosterone. Namely, there was increased activation in the right amygdala during threat approach after testosterone administration [Substance (testosterone > placebo) × Emotion (angry > happy) × Movement (approach > avoid) interaction; region of interest (ROI) analysis: coordinates, 32 −2 −16; $z$ value = 3.63; $P_{FWE}$ ($P$ value with family-wise error correction) = 0.043]. Analyses involving the reversed Emotion contrast (that is, happy > angry) did not yield significant amygdala activation. Post hoc testing confirmed that the motivation effect was specific for angry faces (Fig. 1; Substance × Movement interaction: $F_{1,52}$ = 8.58, $P$ = 0.005, partial $\eta^2$ = 0.14). Compared to placebo, testosterone administration increased amygdala activity during approach trials ($F_{1,52}$ = 6.06, $P$ = 0.017, partial $\eta^2$ = 0.10) but decreased it during avoidance trials ($F_{1,52}$ = 8.68, $P$ = 0.005, partial $\eta^2$ = 0.14). In addition, amygdala activity significantly differed between approach and avoidance of angry faces after testosterone administration ($F_{1,25}$ = 6.33, $P$ = 0.019, partial $\eta^2$ = 0.20) but not after placebo ($F_{1,27}$ = 2.86, $P$ = 0.10, partial $\eta^2$ = 0.09). A similar pattern of activation was also present at an uncorrected threshold within a left amygdala cluster (coordinates, −32 −8 −18; $z$ value = 2.58; $P_{uncorr}$ = 0.005; see Supplementary Methods and Results).

The activity of the aPFC was increased during incongruent compared to congruent responses (Emotion × Movement interaction: coordinates of local maxima, 30 62 −4; $z$ value = 4.30; $P_{FWE}$ = 0.03; see Fig. 2), additionally corroborating the robustness of this task, replicating previous findings (*15*–*17*). These cerebral congruency effects were present under both substances (for placebo: coordinates of local maxima, 26 54 18; $z$ value = 4.36; $P_{FWE}$ = 0.024; for testosterone: coordinates of local maxima, 30 64 −4; $z$ value = 4.64; $P_{FWE}$ = 0.008, respectively). Testing for differential effects of cerebral congruency, that is, interactions between substance and congruency, yielded

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**Table 1. Means (SE) of salivary hormone levels.** *P* values indicate differences between substance groups. For cortisol, there was only a general effect of time, that is, decreasing cortisol levels in the course of the experiment.

| | Placebo | Testosterone | *P* value |
|---|---|---|---|
| Testosterone levels (pg/ml) | | | |
| At baseline | 22.2 (2.8) | 23.6 (3.0) | 0.74 |
| 3 hours after administration | 15.2 (2.0) | 1361.4 (290.1) | <0.001 |
| 5 hours after administration | 21.4 (2.4) | 450.2 (78.8) | <0.001 |
| Cortisol levels (nM) | | | |
| At baseline | 16.7 (2.4) | 14.5 (1.2) | 0.42 |
| 3 hours after administration | 6.6 (1.2) | 5.3 (0.4) | 0.32 |
| 5 hours after administration | 6.1 (0.5) | 5.8 (0.4) | 0.62 |

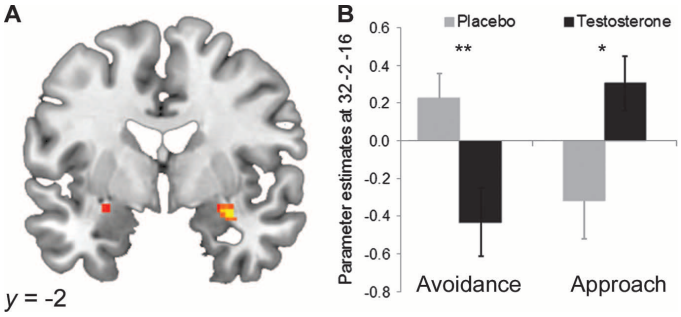


**Fig. 1. Amygdala reactivity (local maxima, 32 −2 −16) showing motivation-specific effects of testosterone during threat approach and avoidance.** (**A**) Enhanced activation for approach versus avoidance of angry faces after testosterone administration compared to placebo. The image is thresholded at $P < 0.05$ (uncorrected) for visualization purposes. Note that a cluster of activation showing a similar pattern is also present at uncorrected threshold within the left amygdala (coordinates, −32 −8 −18; *z* value = 2.58; $P_{uncorr} = 0.005$; see fig. S1). (**B**) Contrast estimates for right amygdala cluster during approach and avoidance of angry faces in each condition. Error bars represent SEM. *$P < 0.05$, **$P < 0.01$.

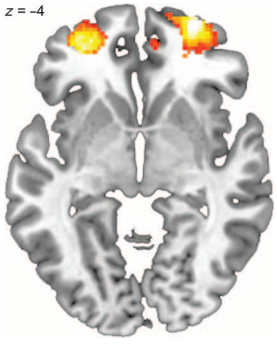


**Fig. 2. Across-group aPFC activity (local maxima, 30 62 −4) for motivationally incongruent versus congruent trials within Brodmann area 10.** The image is thresholded at $P < 0.05$ (uncorrected) for visualization purposes. No other clusters reached significance for the comparison between incongruent and congruent responses in the whole group (that is, none reached whole-brain FWE correction). aPFC activity did not differ between substance groups.

**Table 2. Means (SE) of RTs during the social AA task in milliseconds.** *P* values indicate differences between substance groups.

| | Placebo | Testosterone | *P* value |
|---|---|---|---|
| Happy approach | 676 (29) | 655 (19) | 0.55 |
| Happy avoid | 745 (29) | 692 (22) | 0.16 |
| Angry approach | 723 (29) | 683 (23) | 0.30 |
| Angry avoid | 713 (29) | 680 (20) | 0.36 |

no significant effects in the aPFC. Similarly, there were no testosterone-dependent changes in connectivity between the amygdala and the right aPFC during incongruent compared to congruent responses ($P_{FWE} < 0.05$, seed at right aPFC cluster). Together, these results indicate that the between-group differences in amygdala responses are not due to dissimilar aPFC involvement.

Behaviorally, the social AA task elicited faster approach movements to happy faces and avoidance movements to angry faces (congruent reactions) than when the opposite, incongruent movements needed to be executed [Emotion × Movement interaction in the repeated-measures analysis of variance (ANOVA) on the reaction times (RTs): $F_{1,50} = 28.91$, $P < 0.001$, partial $\eta^2 = 0.36$; see Table 2 for means]. There was an additional main effect of Movement ($F_{1,50} = 29.16$, $P < 0.001$, partial $\eta^2 = 0.36$) being due to faster approach (mean, 684.08; SD, 130.88) than avoidance movements (mean, 707.78; SD, 132.69), as previously reported with this joystick (*17*). None of the substance-related effects were significant (all *F*'s < 2.9 and *P*'s > 0.09), indicating well-matched task performance across substance groups.

## DISCUSSION

This pharmacological fMRI study offers causal evidence that testosterone modulates the amygdala in a motivation-specific manner. Our findings indicate that increased amygdala activity after testosterone administration is bound to social threat approach. Previous studies reported preferential processing of threat after testosterone administration during passive viewing or matching of faces (*3*–*6*). By differentiating between approach and avoidance, we showed that testosterone modulates amygdala reactivity according to current motivational demands and not according to the emotional or action context per se. This motivation-specific mechanism converges with approach-enhancing effects of testosterone observed during social challenges (*12*–*14*).

The present study advances recent motivational salience models (*10*, *11*) by providing evidence for a neuroendocrine mechanism in which testosterone biases the organism toward threat approach and away from threat avoidance by modulating amygdala responses. This enhances our understanding of the processes by which testosterone primes the individual for defense of its status in social challenges. Activational effects of testosterone, such as increased vigilance and up-regulation of neural circuits mediating aggression (*3*–*6*), inhibition of fear responses (*18*), and facilitated threat approach (*12*), further contribute to adaptive responding. In competitive interactions, testosterone has been shown to promote status not only by means of overt aggression but also by more subtle dominance displays, such as increased reciprocity (*20*, *21*).

The motivation-specific effects of testosterone on amygdala tuning were not complemented by behavioral changes, as previously obtained outside a scanner with a more salient AA task (*12*). Building on an extensive line of research applying the current AA task (*15*–*17*), we used a relatively mild task version that is particularly sensitive to isolate neural effects. In line with previous fMRI studies (*15*–*17*), we only found effects on the neural level with task performance being well matched across substance groups. Therefore, we can conclude that the testosterone-induced bias of the amygdala toward threat approach is not due to a neural consequence of behavioral differences.

Alternatively, approaching threat might be perceived as "congruent" after testosterone administration, so that internally driven emotional response tendencies and externally driven task instructions overlap. However, in the current study, there was not a reversal in the direction of behavioral congruency effects after testosterone administration. Moreover, if approaching became more pleasant after testosterone administration, the amygdala activation would reflect a main effect of action direction. No such effect was observed ($P_{FWE} < 0.05$). This supports our finding that the amygdala effects result from the interaction between stimulus valence and action direction.

Our findings may have direct treatment implications for individuals suffering from dysregulations of social AA such as patients with anxiety disorders or depression (*9*). Lower testosterone levels were observed in patients with social anxiety disorder, patients with generalized anxiety disorder, and patients with depression (*22*). The notion that external administration of hormones might compensate for low endogenous testosterone levels and social affective symptoms associated with it remains to be investigated in clinical research.

## MATERIALS AND METHODS

### Study design

**Subjects.** Fifty-four female volunteers (mean age, 21.6; SD, 2.4; range, 18 to 30) were enrolled for participation. Exclusion criteria were history of endocrine, neurological or psychiatric disorder, left-hand dominance, uncorrected vision, habitual smoking, use of medication or drugs (except for paracetamol and contraceptives), current parodontitis, pregnancy or breast-feeding, and irregular sleep patterns.

All participants were using hormonal contraceptives to control for changes in endocrine levels over the menstrual cycle. Testosterone- and placebo-treated subjects did not differ with respect to their personality traits (see table S1). The participants were asked to abstain from alcohol and nicotine 24 hours before testing and received a standardized light lunch on the day of testing. Written informed consent was obtained from all the participants, and the study protocol was approved by the Commissie Mensgebonden Onderzoek (CMO) Nijmegen-Arnhem in accordance with the declaration of Helsinki. All participants received financial compensation.

**Substance administration and procedure.** In a double-blind, randomized, placebo-controlled, between-subjects design, the participants received either a single dose of 0.5 mg of testosterone ($n = 26$; testosterone was suspended in 0.5 ml of solution with 0.5 mg of hydroxypropyl-β-cyclodextrin, 0.005 ml of 96% ethanol, and distilled water) or a matched placebo ($n = 28$) containing the same ingredients except for the testosterone. Both liquids were manufactured by the pharmacy of the Leiden University Medical Center in accordance with good manufacturing practice. The pharmacy was also in charge of blinding and randomization, and the participants were not able to detect which substance they had received above chance level (54% were correct; $\chi^2 = 0.30$, $P = 0.68$).

About 30 min after arrival in the laboratory between 10:00 a.m. and 12:30 p.m., the participants self-administered the liquid under the supervision of the experimenter. Specifically, they held the solution under their tongue for 60 s before swallowing it. This procedure entails the direct absorption of testosterone into the bloodstream, leading to a sharp increase in plasma testosterone 15 min after administration (*23*). Previous research has demonstrated that behavioral and physiological effects are measureable about 4 to 6 hours after testosterone intake (*19*), which has been further confirmed in investigations of social-emotional behaviors in young females [for example, (*18*, *24*–*28*)].

Subsequent to substance administration and before testosterone effects were expected to emerge, the participants filled out several personality questionnaires to characterize and compare the subject sample (see the Supplementary Materials). Additionally, the participants received instructions and training for the experimental tasks. For the remaining time, they were allowed to do schoolwork or reading in the waiting room, but social interaction was restricted. Four hours later, the participants were positioned in the MRI for an anatomical scan (6 min), the social AA task (20 to 25 min), and another experimental task (20 min; reported elsewhere). Thus, the data reported here were obtained in the afternoon, that is, after 1:30 p.m., between 4 and 4.5 hours after testosterone administration. After the MRI session, the participants completed several exit questionnaires, for example, assessing their belief on whether they received testosterone or placebo, and were debriefed. Saliva samples for cortisol and testosterone analyses were obtained at baseline (upon arrival in the laboratory) and 30 min, 3 hours, and 5 hours after administration. The total duration of the experimental session was 6.5 hours.

**AA task.** In this fMRI-adapted RT task (*16*, *29*), the participants had to respond to visually presented emotional facial expressions by either pulling a joystick toward their body (approach movement) or pushing it away from their body (avoidance movement). Stimuli were taken from several databases (*30*–*33*) and contained two affective expressions (happy and angry) for each of the 36 models (18 females). The faces were trimmed to exclude influences from hair and nonfacial contours (*34*) and matched for brightness and contrast values.

The task comprised of 16 blocks with 12 trials per block in which the participants had to categorize the affective expression. At the start of each block, participants received written instructions regarding the stimulus-response mapping, that is, either pulling the joystick upon seeing a happy face and pushing for an angry face (affect-congruent trials), or pushing for a happy face and pulling for an angry face (affect-incongruent trials). This operationalization is in line with a recent meta-analysis (*35*) demonstrating strongest effects for the explicit evaluation of the affective value of stimuli (happy/angry). The mapping changed after each block and its order was counterbalanced across participants. Blocks were separated by an interblock interval of 21 to 24 s.

Each trial started with a blank screen for 300 ms. Subsequently, the stimulus was presented in grayscale against a black background for 100 ms, followed by the participants' response and a variable intertrial interval (ITI; blank screen; 2 to 4 s). Following previous studies using the AA task during fMRI (*15*–*17*), images were presented without a "zooming" feature to avoid neural confounds related to an image moving toward or away from the participants as well as mere exposure effects.

Valid responses were defined as joystick displacements of at least 80% along the sagittal plane occurring within 2 s after stimulus presentation. After their response, the participants had to move the joystick back to the starting position (the central area of 20% on the sagittal plane) before the end of the ITI. The participants received feedback in the case of kinematically invalid responses ("You did not move the joystick far enough."; "Please return the joystick to the starting position.").

Stimuli were projected (visual angle, 4° × 6°) at the center of a screen that was viewed via a mirror above the participants' head. An MR-compatible joystick (Fiber Optic Joystick, Current Designs; sampling rate, 550 Hz) was positioned on the abdomen of the participants. Presentation of stimuli and acquisition of responses were controlled by a PC running Presentation software version 13.

**Image acquisition.** Images were acquired on a 1.5-T MRI scanner (Avanto, Siemens Medical Systems) equipped with an eight-channel head coil using a multiecho GRAPPA (GeneRalized Autocalibrating Partially Parallel Acquisitions) sequence (*36*) [repetition time (TR), 2.14 ms; echo times (TEs), 9.4/21/33/44/56 ms; 34 transversal slices; ascending acquisition; distance factor, 17%; effective voxel size, 3.3 × 3.3 × 3.5 mm; field of view (FoV), 212 mm]. Anatomical images were acquired using an MP_RAGE (magnetization-prepared rapid gradient-echo) sequence (TR, 2250 ms; TE, 2.95 ms; 176 sagittal slices; voxel size, 1.0 × 1.0 × 1.0 mm; FoV, 256 mm).

**Salivary hormone measures.** Saliva was collected in 15-ml Cellstar tubes (Greiner Bio-One) and stored at −25°C. Samples were analyzed in duplicate for testosterone and cortisol, and the average was used in subsequent analyses. Hormone concentrations were measured using Luminescence Immunoassays (Immuno-Biological Laboratories GmbH). The average intra-assay and interassay coefficients were between 4.8 and 7.8% for cortisol and 6.5 and 8.6% for testosterone.

## Statistical analyses

**Behavioral data.** Trials with incorrect or no responses were classified as errors and analyzed separately. The error rate was calculated per level of the two experimental factors per participant. When the error rate in a block was above chance level, the whole block was excluded because it can be expected that the participants misunderstood the instructions for that specific block (*15*–*17*). In total, 11.1% of trials were excluded (of which 5.3% were errors, 0.9% were omissions, 0.4% were excluded because of block errors, and 4.5% were anomalous kinematic responses), with 9.98% excluded in the testosterone group and 11.59% in the placebo group, which is comparable to previous studies with this paradigm (*16*, *17*). Groups did not differ in the number of excluded trials ($t_{52} = 0.95$, $P = 0.35$).

The time from stimulus presentation until movement onset corresponds to movement initiation, and the time from movement onset until reaching the target position of the joystick reflects the movement duration. The RT was defined as the time from stimulus presentation until attainment of the target position. Trials with a movement duration shorter than 400 ms and those with a movement initiation outside the range of 100 to 1500 ms or exceeding 3 SDs from the subject-specific mean were excluded. Median RTs were calculated for each level of the two experimental factors (Emotion and Movement) and subjected to a repeated-measures ANOVA, with the within-subject factors Emotion (angry and happy) and Movement (approach and avoid) and the between-subjects factor Substance (testosterone and placebo).

Independent *t* tests were used to assess group difference on baseline levels of testosterone and cortisol. The standardized testosterone and cortisol levels from the first saliva measurement were included in the ANOVAs as covariates. For all analyses, the α level was set at $P < 0.05$. For the ANOVAs, within-subject effects with Greenhouse-Geisser correction are reported. Statistical testing was performed with the Statistical Package for the Social Sciences (IBM SPSS 19).

**Imaging data.** Statistical parametric mapping (SPM8) was used for preprocessing and analyzing the imaging data. To allow for magnetic saturation, the first four volumes of each data set were discarded. Using a least-squares approach with six rigid body transformation parameters (translations and rotations), motion parameters were estimated on the basis of the MR images of the first echo (TE, 9.4 s) (*16*). After applying the motion correction parameters to the images from all echoes, single MR volumes were obtained by combining the five echo images based on an optimized echo weighting method (*36*). Subsequently, images were slice time–corrected, and the anatomical scan was co-registered with the mean of the functional images. Normalization into Montreal Neurological Institute space was based on a segmentation algorithm (*37*). Images were resampled at a voxel size of 2 × 2 × 2 mm and spatially smoothed using an 8-mm full-width-at-half-maximum Gaussian kernel.

The general linear model was applied to the time series of each participant. For this event-related design, trials were averaged separately for each condition, yielding the following four task-relevant regressors: approach-happy, avoid-happy, approach-angry, and avoid-angry. Two additional regressors were derived from modeling missed responses and periods where instructions were presented. Events were isolated by convolving vectors of stimulus onset times and movement initiation as duration with the canonical hemodynamic response function. Potentially confounding residual head movement effects were modeled with regressors based on the original, squared, cubic, first-order, and second-order derivatives of the movement parameters (*38*) as well as signal intensities of white matter, cerebrospinal fluid, and the portion of the MR image outside the skull. Finally, images were high- pass–filtered at 128 s, and an autoregressive AR(1) model was used to account for serial correlations in fMRI time series.

Analyses on the group level were performed by subjecting the four task-relevant contrast images per participant (approach-happy, avoid-happy, approach-angry, and avoid-angry) to a random-effects multiple regression analysis (Substance × Emotion × Movement). Standardized endogenous testosterone and cortisol levels (obtained from the first saliva sample) were included as covariates, resulting in another 16 regressors [as in, for example, (*16*)]. The following effects were considered.

First, to address our main question of whether testosterone administration modulates amygdala reactivity to angry faces, we performed a ROI analysis on the amygdala (comprising the basolateral, corticomedial, and superficial subregions bilaterally) based on cytoarchitectonic probability maps implemented in the SPM toolbox (*39*, *40*). Here, we tested for the three-way interactions of Substance × Emotion × Movement. To understand potential interaction effects, parameter estimates were extracted from the activation cluster within the amygdala and subjected to statistical analyses in SPSS with α set at $P < 0.05$.

Second, to examine the involvement of the aPFC in motivationally incongruent responses irrespective of hormonal modulations, the congruency effect (Emotion × Movement) was assessed for both groups together and separately with an ROI based on Brodmann area 10, which has previously been implicated during this task (*15*–*17*). In other

words, activation during affect-incongruent trials (approach-angry and avoid-happy) was compared to that on affect-congruent trials (approach-happy and avoid-angry). The reported activations from the ROI analyses are corrected for multiple comparisons over the search volume using FWE ($P < 0.05$) (*41*).

An additional psychophysiological interaction (PPI) analysis was conducted to explore whether there was a task- and testosterone-dependent modulation in connectivity between the amygdala and right aPFC during incongruent versus congruent trials, using an 8-mm sphere around the right aPFC (30 62 −4; see Results) as a seed region. Within this region, voxels showing task-related activity ($P < 0.01$, uncorrected), assessed by an *F*-contrast of task-related regressors (approach-happy, avoid-happy, approach-angry, and avoid-angry), were included. Participants who did not show any voxels with significant task-related activity in this region were excluded from the PPI analysis ($n = 5$, of which $n = 2$ in the testosterone group and $n = 3$ in the placebo group). Subject-specific contrast images of the remaining 49 participants describing the PPI between the time course of the aPFC volume of interest (VOI) and the time course of the incongruent versus congruent trials were generated. Group inferences were based on a multiple regression analysis with the participant-specific contrast images and their endogenous testosterone and cortisol levels (obtained from the first saliva sample) as regressors. Apart from whole-brain analyses, we tested for substance differences within an amygdala VOI that was created on the basis of the activation identified in the ROI analyses of approach versus avoidance of angry faces (see Results) using a small-volume correction (*41*) ($P_{FWE} < 0.05$).

## SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/1/5/e1400074/DC1

Fig. S1. Contrast estimates for the left amygdala cluster during approach and avoidance of angry faces in each condition.

Table S1. Means (SE) of questionnaire scores.

Methods and Results. Motivation-specific effects of testosterone during threat approach and avoidance within the left amygdala.

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