Promoting social behavior with oxytocin in high-functioning autism spectrum disorders

Elissar Andari[a], Jean-René Duhamel[a], Tiziana Zalla[b], Evelyn Herbrecht[b], Marion Leboyer[b], and Angela Sirigu[a,1]

[a]Centre de Neuroscience Cognitive, Unité Mixte de Recherche 5229, Centre National de la Recherche Scientifique, 69675 Bron, France; and [b]Institut National de la Santé et de la Recherche Médicale U 841, Department of Psychiatry, Hôpital Chenevier-Mondor, 94000 Créteil, France

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Social adaptation requires specific cognitive and emotional competences. Individuals with high-functioning autism or with Asperger syndrome cannot understand or engage in social situations despite preserved intellectual abilities. Recently, it has been suggested that oxytocin, a hormone known to promote mother-infant bonds, may be implicated in the social deficit of autism. We investigated the behavioral effects of oxytocin in 13 subjects with autism. In a simulated ball game where participants interacted with fictitious partners, we found that after oxytocin inhalation, patients exhibited stronger interactions with the most socially cooperative partner and reported enhanced feelings of trust and preference. Also, during free viewing of pictures of faces, oxytocin selectively increased patients' gazing time on the socially informative region of the face, namely the eyes. Thus, under oxytocin, patients respond more strongly to others and exhibit more appropriate social behavior and affect, suggesting a therapeutic potential of oxytocin through its action on a core dimension of autism.

cognition | neurodevelopmental disorder | recovery | Syntocinon

Our brain is endowed with the ability to detect and respond to simple social signals such as eye contact, as well as to infer from more complex behaviors intrinsically social qualities of other people such as fairness or cooperation. Individuals suffering from high-functioning autism spectrum disorders (HF-ASD), a neurodevelopmental disorder, are impaired in understanding social cues and in responding to them. These patients generally have normal language or general intellectual abilities, yet in everyday life they avoid eye contact (1–3) and do not spontaneously interact with people (4). On formal tests of social cognitive skill, they show specific impairments in understanding the intentions of others (1, 5) and lack of fast intuitive judgments about social contexts (4).

The pathogenesis of autism is unclear, although mutations in genes implicated in synaptogenesis have been identified (6, 7) and different neurochemical, neurophysiological, and neuropathological abnormalities have been demonstrated in these patients (8). An interesting current hypothesis has implicated oxytocin in the etiology of autism, and in particular in the social disorders that are the hallmark of HF-ASD (8–10).

Oxytocin is a hormone synthesized in the hypothalamus. Best known for its facilitatory role in parturition and lactation, oxytocin is also involved in the regulation of emotions and has receptors distributed in various brain regions including the limbic system and amygdala (11, 12). In mammals, it has been associated specifically with the development of prosocial behavior such as mother-infant attachment, grooming, approach behavior, sexual activity, and stress regulation (13, 14). Oxytocin anomalies have been reported in children with autism. They have significantly lower plasma oxytocin levels compared to control subjects (15) and fail to show the normal developmental increase in oxytocin blood levels. Moreover, plasma samples are associated with higher oxytocin precursor levels, suggesting that autism may be related to anomalies in the way this hormone is synthesized (16).

Experimental manipulation of brain oxytocin levels in healthy human subjects confirms its involvement in the expression of human affiliative social behavior (17). In a simulated economic investment game, subjects who received an intranasal spray of oxytocin were more inclined, as compared to a placebo control group, to trust another player by sending him money with no guarantee of reciprocation, suggesting that oxytocin acts on brain circuits that promote social proximity and affiliation with peers (17). Recently, it has been shown that oxytocin facilitates recognition of memorized faces and strengthens the encoding of social stimuli (18, 19). Moreover, oxytocin has been reported to increase the time spent looking at socially important cues, such as the eyes, when viewing pictures of human faces (20). In the light of the above findings, a key question regarding both the role of oxytocin in the nervous system and the pathophysiology of social disorders in autism is whether administration of oxytocin can influence social interaction behavior in individuals with autism. We investigated the effects of intranasal oxytocin on the social behavior of 13 patients suffering from HF-ASD and compared these effects to a placebo condition and to the behavior of matched healthy subjects. Two different behavioral measures were used: (*i*) decision making and affect in a social interaction game, and (*ii*) eye movement recordings during a face perception task. We also measured plasma oxytocin levels in patients before and after nasal spray intake, to establish whether patients displayed physiological abnormalities in oxytocin and to verify the effectiveness of the nasal administration procedure in enhancing plasma oxytocin levels.

Results

Social Ball Tossing Game. We used a social interaction task inspired by the Cyberball game (21) in which the participant engages in a multiround ball-toss game over a computer network with three fictitious partners (Fig. 1*A Inset*). In our variant game, we manipulated the amount of reciprocation exhibited by the three fictitious players. The critical task manipulation was the probability that each of the three fictitious players would throw the ball to the participant, which allowed us to create different cooperative behavior profiles (good, bad, and neutral) (*Materials and Methods* and *SI Materials and Methods*).

Oxytocin Effect on Social Decision. The behavioral decision variable of interest in this task is the participant's ball-toss choices. Under placebo treatment, patients showed little evidence that they discriminated the three players' cooperative profiles. Whereas healthy subjects sent significantly more balls to the good than to the bad (Wilcoxon test, $z = 3$, $P < 0.003$) or neutral player ($z =$

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[1]To whom correspondence should be addressed. E-mail: sirigu@isc.cnrs.fr.

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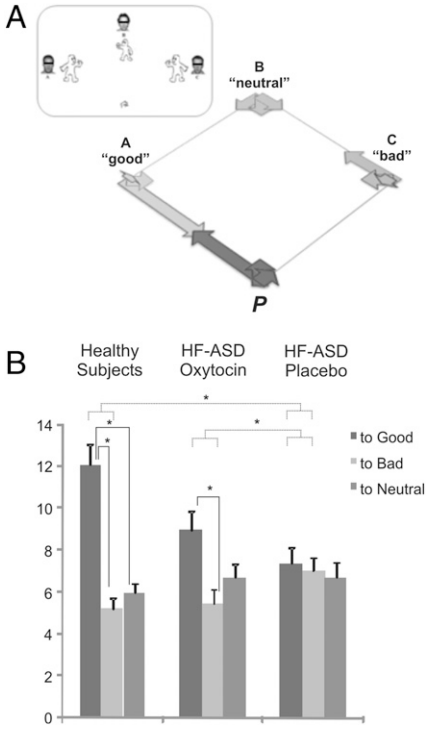

Fig. 1. Cyberball game and ball-toss distributions toward each of the three partners. (*A*) Schematic representation of the modified Cyberball game. On successive trials, the role of the participant *P* alternates between turns as observer of ball exchanges between two of the other players, as ball recipient, and as ball sender. The behavior of players A, B, and C is computer-generated so as to define three different profiles from *P*'s standpoint: an includer or good profile, a neutral profile, and an excluder or bad profile. The length of the gray arrows is proportional to the number of balls sent by a given player to each of the other players. The profiles represent the average behavior over the entire game but, rather than being fixed from the beginning, they were tuned progressively using an algorithm described in *SI Text*. Black arrows represent the behavior of a representative healthy subject. (*B*) Ball-toss distributions for healthy subjects and for patients with HF-ASD treated with oxytocin or placebo. Under oxytocin, there was a nearly significant trend in the number of balls sent toward the good as compared to the neutral player (significant trend, $z = 1.82$, $P = 0.06$; two-tailed) (middle) (mean and SEM; * indicates significant difference at $P < 0.05$ or better on posthoc pairwise comparisons).

2.76, $P < 0.005$) (Fig. 1*B Left*), patients under placebo responded in the same manner to all players ($z = 0.36$, $P = 0.72$; $z = 0.2$, $P = 0.84$) (Fig. 1*B Right*). In striking contrast, oxytocin intake led patients to engage more often with the good player and to send significantly more balls to this player as compared to the bad one ($z = 2.04$, $P < 0.041$; two-tailed) (Fig. 1*B Middle*). When comparing directly the effects of placebo and oxytocin, we also found a significantly larger difference in the number of balls sent to the good versus the bad player in the oxytocin condition ($z = 1.99$, $P < 0.047$; two-tailed). Finally, the difference in performance (number of balls sent to the good versus the bad player) between the control subjects and patients, which was significant under placebo (Mann–Whitney *U* test: $z = 3.1$, $P < 0.0021$), disappeared when the comparison was made with the oxytocin treatment condition ($z = 1.62$, $P = 0.11$).

A finer-grained image of the patients' decision making was obtained by examining the distribution of ball tosses over time. Data were binned with respect to intervals defined by player A's turns, as it was through the observation of A's behavior that the participant could learn to cooperate with him more than with the other two players. The first six tosses by A were unbiased; hence the probability of the participant receiving the ball $p[A \rightarrow P] = 0.33$. On the next four tosses, A increased the proportion of balls sent to the participant up to $p[A \rightarrow P] = 0.50$. From the 11th toss on, A sent all of its balls to *P*, that is, $p[A \rightarrow P] = 1.0$. Over the same period, player C started to exclude *P*, revealing himself as the bad player. The effect of these biases on the behavior of the participants is illustrated in Fig. 2. Because preliminary analyses in both healthy subjects and patients failed to reveal any differences in behavior toward the neutral versus the bad player, we focus on good and bad players only. Both healthy subjects and patients under oxytocin begin to cooperate preferentially with the good player at about the same time, with the cumulative number of balls sent to the good and bad players diverging significantly in the 15–17 interval (Fig. 2 *A* and *B*; first of two consecutive significant bins for the difference between good and bad, healthy subjects: $z = 2.7$, $P < 0.007$; oxytocin: $z = 2.04$, $P < 0.041$; two-tailed, Wilcoxon test). By contrast, under placebo, the patients' cumulative ball-toss curves never diverged significantly (Fig. 2*C*).

Oxytocin Effect on Emotions. The emotional response of the patients to the fictitious players' personality was assessed after completion of the task using a seven-point rating scale. These emotional self-ratings were consistent with their decision behavior under both treatment conditions. Whereas feelings (trust and preference) expressed toward the three fictitious players did not differ in the placebo condition (Friedman's ANOVA, respectively: $\chi^2 = 2.39$, $P = 0.3$; $\chi^2 = 1.19$, $P = 0.55$), patients reported that they trusted more and showed stronger preference for the good than the bad player after playing under oxytocin (Friedman's ANOVA, respectively: $\chi^2 = 17.89$, $P < 0.0002$; $\chi^2 = 13.63$, $P < 0.001$; posthoc pairwise comparisons $P < 0.05$; Fig. 3). No significant differences were found between feelings toward the neutral and the other two players.

One question which could be raised about the effect of oxytocin on ball-toss choices is whether it mainly acted on social engagement or on the perception of monetary rewards. To address this issue, we tested a new group of seven HF-ASD patients on the same ball-toss game but modified the contextual framing of the task to eliminate any reference to monetary incentives. The task conditions and oxytocin administration procedures were exactly the same as in the original version except that subjects were instructed that the goal of the task was to play a friendly ball-toss game with other players, but no monetary reward was promised and the participant did not receive any feedback about the number of balls he/she received. They were only told that whenever they tossed the ball to someone, that player could either send it back or toss it to another player. Following completion of the task, the participants again estimated their feelings of "trust" and "preference" with respect to the fictitious players. Despite the smaller size of the patient sample, we again found a significant, positive effect of oxytocin on the participant's capacities to discriminate between the two extreme player profiles (Fig. S1). Comparing directly the effects of placebo and oxytocin, we found a significantly larger difference in number of balls sent to the good versus the bad player in the oxytocin condition ($z = 1.99$, $P < 0.047$; two-tailed) (Fig. S1). Consistent with this behavior, patients also reported that they felt more trust toward the good than the bad player under oxytocin ($z = 2.11$, $P < 0.035$; two-tailed), whereas under placebo, there was no such difference ($z = 0.59$, $P > 0.58$). In the oxytocin condition, a similar trend was found in the feelings of preference but it did not reach significance ($z = 1.57$, $P = 0.11$).

Visual Scanning of Faces. To strengthen our observations of the effects of oxytocin on the processing of socially relevant information, we investigated how patients looked at a fundamental social stimulus, such as human faces. Participants examined

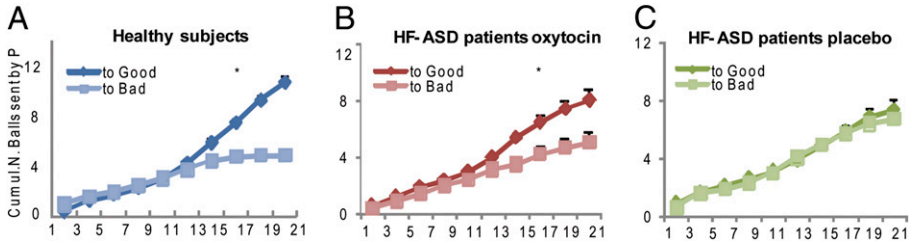

Fig. 2. Time course of ball tosses during the Cyberball game. Cumulative number of balls sent by the participant P to players A (good) and C (bad) in regularly spaced bins, for healthy subjects and for patients with HF-ASD under oxytocin and under placebo. Each data point falls in a bin defined by an interval between player A's tosses *n* and *n+2*. The participant had, on average, three ball-toss opportunities in each interval. The transition in player A's behavior from unbiased (equal probability of throwing the ball to each player) to positively biased toward P (100% probability of throwing the ball to P) was progressive (mean and SEM; * indicates significant difference at $P < 0.05$ or better for the first of two significant consecutive bins).

pictures of faces presented one at a time on a computer monitor while their eye movements were being recorded. The participants' task was to report either the gender (male/female) or the gaze direction (direct/averted) of the depicted face (Fig. S2). Offline, we computed the total fixation time inside each of six regions of interest (eyes, nose, mouth, forehead, cheeks, and outside of facial contour) and the number of saccades (rapid displacements of the line of gaze) elicited by the face stimuli.

Normal subjects directed their gaze preferentially within the contours of the faces [gender identification (GI): 80% ± 2.09; gaze direction (GD): 84% ± 1.74; Tables S1 and S2]. By contrast, patients under placebo spent significantly less time looking directly at the faces as compared to healthy subjects (53% ± 7.2 in GI: Mann–Whitney *U* test, $z = 2.67$, $P < 0.0077$; 41% ± 8.04 in GD: $z = 4.08$, $P < 0.00005$). More detailed analysis of scanning pattern by regions of interest shows that they specifically avoided the eye region ($z = 2.88$, $P < 0.004$; $z = 3.48$, $P < 0.0005$ for GI and GD, respectively). Interestingly, during gaze direction judgments, patients also produced more saccades than healthy subjects (GD: $z = 2.45$, $P < 0.015$). This increase in saccade rate was only present during epochs when patients looked at faces directly and not when they explored the rest of the display (GD: $z = 0.71$, $P > 0.47$). Such underexploration of the face and eyes, in association with high saccade frequency, implies that patients explored these images hastily by means of multiple brief fixations, probably with high levels of anxiety and discomfort. Saccade frequency was not increased during gender decisions (GI: $z = 1.36$, $P > 0.17$), possibly because, in contrast to gaze direction judgment, it does not depend critically upon attending to the eye region of the faces.

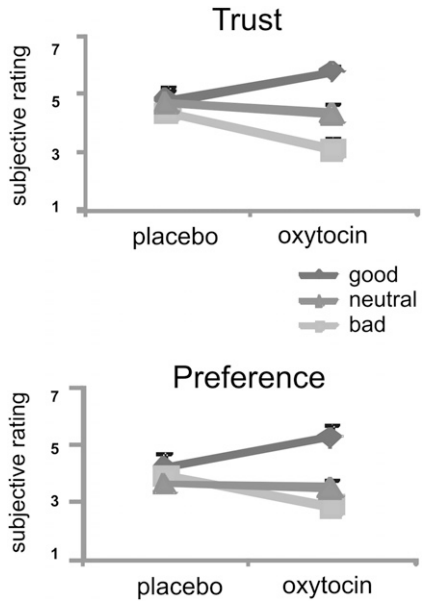

Fig. 3. Subjective postexperimental rating under oxytocin and placebo treatment. Rating (1–7) of subjective feeling states toward the three players in the placebo and oxytocin-treated patients with HF-ASD (mean and SEM; * indicates significant difference at $P < 0.05$ or better on posthoc pairwise comparisons).

Oxytocin modified how patients responded to pictures of faces, as compared to the placebo condition. Total gaze time over the face increased significantly under both task conditions (GI: $z = 2.27$, $P < 0.023$; GD: $z = 2.19$, $P < 0.029$; two-tailed Wilcoxon test; Fig. 4 *A* and *B Left*). Broken down by region of interest, the effects of oxytocin are found to be largely accounted for by an increased fixation time over the eye region (GI: $z = 2.12$, $P < 0.04$; nearly significant trend for GD: $z = 1.88$, $P = 0.059$; Fig. 4 *C* and *D*). No effects of oxytocin were observed over the other regions of interest (mouth + nose, GI: $z = 1.18$, $P > 0.23$; GD: $z = 1.41$, $P > 0.15$; forehead + cheeks, GI: $z = 39$, $P > 0.69$; GD: $z = 0.86$, $P > 0.38$) (Fig. 4 *C* and *D Right*). Finally, oxytocin significantly reduced the abnormally high saccade frequency observed under placebo during gaze direction judgments ($z = 2.12$, $P < 0.03$; two-tailed; Table S2).

Although oxytocin significantly enhanced patients' visual scanning of faces, as compared to the placebo condition, their gaze time on the face and eye region remained significantly lower than that of healthy subjects for all comparisons with the exception of whole-face scanning in the gender identification condition (Mann–Whitney *U* test; face: GD: $z = 3.21$, $P < 0.002$; GI: $z = 1.96$, $P > 0.05$; eye: GI: $z = 2.77$, $P < 0.006$; GD: $z = 2.99$, $P < 0.003$). The fact that oxytocin did not fully restore a normal visual exploration pattern in patients is discussed below in relation to the magnitude of the changes in blood oxytocin.

In summary, under oxytocin, patients with HF-ASD spent more time looking at the face pictures and, specifically, at the eye region. The accompanying decrease in saccade frequency, that is, the increase in the average duration of individual fixations, suggests that oxytocin may reduce the fear or anxiety induced by face stimuli in these patients.

We tested for a possible effect of treatment order by comparing patients' performance between the two visits in the placebo and the oxytocin condition. No differences were found between the two visits on any of the dependent variables measured in the ball-tossing and face-scanning tasks (Mann–Whitney *U* test; $z = 0$, $P = 1$). We also found that oxytocin's effect on patients' performance during both tasks was not related to a simple mood effect (see *SI Results*).

Plasma Oxytocin Levels. Baseline plasma oxytocin concentration in patients (1.08 pg/mL ± 1.04) was significantly below the values observed in a normative group of healthy subjects (7.28 pg/mL ±

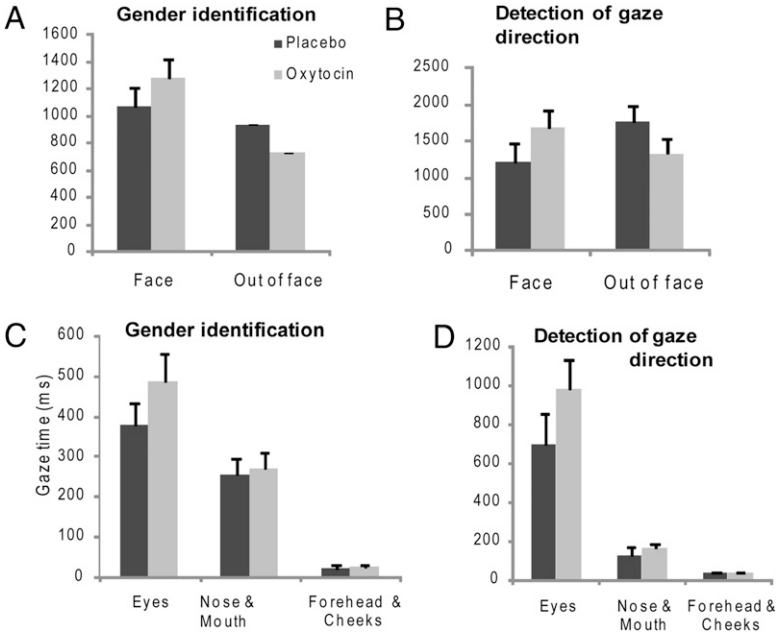

Fig. 4. Mean gaze time spent on different regions of interest for patients with HF-ASD under placebo and oxytocin treatment. (*A* and *B*) Gaze time spent on the face and outside the face region under oxytocin and placebo when patients had to identify the face's gender (male/female) and face's gaze direction (direct/averted), respectively. (*C* and *D*) Gaze time spent on main regions of interest: the eyes, nose and mouth, and other regions such as forehead and cheeks during gender identification and gaze direction detection, respectively. * indicates significant difference at $P < 0.05$ or better on Wilcoxon test.

4.49) (Mann–Whitney U test; $z = 4.69$, $P < 0.0001$). A second measurement made 10 min after nasal administration of a dose of 24 IU of Syntocinon spray showed a significant increase in plasma oxytocin concentration (2.66 pg/mL ± 2.2) (Wilcoxon test; $z = 1.88$, $P < 0.02$; Fig. S3), indicating successful assimilation of the substance (*SI Materials and Methods*).

Individual Variability in Response to Oxytocin. Although the social symptoms of HF-ASD can be diagnosed on the basis of well-established, reliable criteria, it is unclear whether these symptoms are related to a common etiological process. A degree of heterogeneity in responsiveness to oxytocin treatment can thus be expected in such a patient group. Inspection of individual performances revealed that some patients responded strongly to oxytocin, others more weakly, and some not at all (Table S3). Furthermore, oxytocin effects on the social game and on the face perception tasks were only weakly correlated (GI: $r = 0.23$; GD: $r = 0.54$, $P > 0.05$), indicating that the two tasks are sensitive to different aspects of social information processing. Indeed, although explicit social engagement is required in the ball game, visual inspection of facial stimuli may involve more implicit, automatic mechanisms. Also, looking directly at large face stimuli may be more threatening to some patients than interacting with other people via a computer network, whereas for others, the dynamical aspect of the social interaction may be more difficult to apprehend than the simple perceptual decision required by the face task. Possible relationships between oxytocin effects and general clinical data on patients were investigated. We found no significant correlation between patients' performance with the Autism Diagnostic Interview-Revised (ADI-R) (ball game: $r = -0.31$; GI: $r = 0.03$; GD: $r = 0.35$; all $P > 0.05$), IQ (ball game: $r = 0.43$; GI: $r = -0.43$; GD: $r = -0.24$; all $P > 0.05$), or age (ball game: $r = 0.25$; GI: $r = -0.2$; GD: $r = -0.09$; all $P > 0.05$). Different authors have suggested that patients with autism may display different social interaction styles (15, 22). According to these authors, one can distinguish between "aloof" individuals who avoid physical proximity with others and actively reject social contact, "passive" individuals who do not reject approaches but neither engage in social relations, and "active-but-odd" individuals who display approach behavior but in a somewhat inappropriate or one-sided manner. We examined whether such qualitative differences in social interaction profiles might account for the variability in the response to oxytocin treatment. Patients in our study were assigned to one of those three categories based on clinical records and parent interviews (6 patients were classified as aloof, 7 as active-but-odd, none as passive). Interestingly, 6/8 patients who showed positive changes on the ball game under oxytocin had been labeled as active-but-odd, whereas 4/5 who showed no positive change were of the aloof type.

Discussion

In this study, we investigated whether oxytocin could modify how high-functioning autistic patients process social signals and social feedback. Oxytocin was shown to enhance visual scanning of faces and, in particular, of the eye region, as compared to a placebo condition. Eye contact between individuals can be considered a basic form of social aptitude. Previous studies in normal individuals indicate that oxytocin enhances processing of facial stimuli (20) and the ability to infer others' mental states from the eye region (23). Here we further demonstrate that oxytocin promotes a first level of prosocial approach by overturning what constitutes a core deficit of patients with HF-ASD, namely the lack of eye contact. How does oxytocin facilitate patients' prosocial behavior? The present data provide some suggestions of the neural mechanisms mediating these effects. It has been proposed that oxytocin enhances affiliation partly by reducing fear of social unfaithfulness and by suppressing avoidance behavior (14) and that it reduces the activity of the amygdala, resulting in a decrease of fear responses (14, 24, 25). It is possible that patients with autism possess latent social skills and that oxytocin may thus favor social engagement behavior by suppressing fear and mistrust.

The results from the ball-tossing task suggest the possibility of other mechanisms underlying the effects of oxytocin on social interaction. In this simple game simulating social exchanges,

patients tested under placebo conditions did not take into account the behavior of other players and showed no differential emotional responses to the different players. By contrast, under oxytocin, these patients engaged more often in exchanges with the player who reciprocated strongly, less often with the player who reciprocated weakly, and they exhibited emotional responses congruent with this behavior. Thus, oxytocin enhanced patients' ability to process socially relevant cues and acquire their meaning in an interactive context. A study conducted in normal subjects showed that oxytocin increases trust of others in the absence of any certainty of reciprocation (17), hinting at the possibility that oxytocin may promote indiscriminate prosocial behavior and "blind" trust. The task used here to study the effects of oxytocin on autistic social difficulties was different in that it involved multiple iterations in which the participant was presented with successive feedback from partners that were endowed with different reciprocating tendencies. Therefore, the behavior exhibited by the participants could evolve over time through a learning process which can be interpreted within a social reinforcement learning framework, with social inclusion acting as reinforcer. The fact that oxytocin allowed recognition of the partner who was willing to reciprocate the most cannot be explained only in terms of prosocial attitudes such as reduced fear or increased approach and trust. The patients' ability to discriminate between the good and bad partners shows that oxytocin facilitated learning, which may in turn result from an increased drive for social affiliation or from an enhancement of reinforcers satisfying this drive. This hypothesis is consistent with data from animal and human studies. Animal studies show that oxytocin promotes social bonding behavior. In rodents, oxytocin has been reported to enhance social recognition, as indicated by a decrease of exploration behavior toward a conspecific during a second encounter (13). Moreover, in oxytocin knockout mice, social memory is impaired but recovered after a single shot of the hormone before initial social encountering takes place. Finally, in humans, different studies have shown that oxytocin improves recognition memory of social relevant cues (19) (i.e., faces) and memory of positive social information (i.e., happy faces) (18).

One question that can be raised is whether oxytocin mainly acted by enhancing sensitivity to social rewards (i.e., to being sent the ball, a social engagement gesture) or by enhancing sensitivity to the accompanying nonsocial reward (the monetary value of the received ball). Motivation for money could not have been the main factor determining their choices. Both healthy subjects and patients were biased toward adopting a prosocial attitude because they sent fewer balls to the good player and more balls to the bad player than would be predicted by an optimal reward-seeking strategy such as the matching law (26). More direct evidence that, in this task, oxytocin is acting on social motivation comes from a second ball-toss game that was performed by an independent group of HF-ASD patients, in which ball exchange was not associated with monetary reward. Also in this case, oxytocin enhanced the propensity to interact with the reciprocating partner, as compared to placebo. This is in keeping with results obtained in a similar social task showing that normal subjects preferred to avoid being excluded from the game even when, as a consequence, they ended up *losing* money (27).

Although previous studies have shown that oxytocin can reduce repetitive behavior in subjects with autism (28) and enhance the comprehension of affective speech (29), here we demonstrated that oxytocin can promote social approach and social comprehension in patients with autism. Individual variability was observed in the effects of oxytocin on the social tasks used in this study. Nevertheless, the results from the ball-toss game were statistically robust and found in two independent groups of HF-ASD patients. More work will be needed to understand the relationship between changes in social behavior induced by oxytocin administration in individuals with autism and local changes in brain oxytocin metabolism. This could be accomplished using functional imaging techniques. Finally, our results highlight the therapeutic potential of oxytocin through its action on core deficits of patients with HF-ASD such as affiliation and cooperative behavior. Although the effect we measured here is certainly transient, it serves to show that these patients are quite able to engage in social relationships. Future research is necessary to investigate whether a long-term intake of oxytocin may improve real-life social functioning of these patients.

Materials and Methods

Participants. A group of 13 adults (11 men and 2 women, mean age = 26, range = 17–39) with a clinical diagnosis of Asperger syndrome (AS) (n = 10) or high-functioning autism (HFA) (n = 3) according to Diagnostic and Statistical Manual-Revision 4 (DSM-IV R) (American Psychiatric Association, 2000) and ASDI (Asperger Syndrome Diagnostic Interview) (30) were recruited from the expert centers (Foundation FondaMental), Chenevier-Mondor Hospital in Créteil, France. Interviews with parents or caregivers using the ADI-R (Autism Diagnostic Interview-Revised) (31) (Table S4) confirmed the diagnoses. As part of the checking process, the French translation of A-TAC (autism, tics, AD-HD, and other comorbidities) (32) was completed by the parents. Patients received verbal and performance IQ tests (WAIS-III) and all showed average to above average estimates of intelligence (Table S4). Patients were medication-free for at least 2 weeks before and throughout the study (*SI Materials and Methods*). A second group of patients was recruited to test oxytocin effects on a social ball-toss game involving no monetary incentives. It included seven new HF-ASD patients (7 men, mean age = 28, range = 18–38; verbal IQ: 96 ± 15.85, performance IQ: 87 ± 20.57, total IQ: 92 ± 17.47; ADI-R: social interaction 12.6 ± 7.21, communication 6.7 ± 3.73, repetitive behaviors 3.1 ± 2.03) with a clinical diagnosis of AS (n = 4) or HFA (n = 1) or pervasive developmental disorder-not otherwise specified (PDD-NOS) (n = 2). The study also included a control group of 13 healthy subjects matched for chronological age and sex to the patients (11 men and 2 women, mean age = 26, range = 18–40). The study was approved by the Local Ethical Committee (Centre Léon Berard, Lyon IV). The French Agency (Agence Française de Sécurité Sanitaire des Produits de Santé) competent for clinical trials on a medicinal product for human use also gave its approval.

Behavioral Experiments. ***Social ball-tossing game.*** During this variant version of the Cyberball game, three players depicted by cartoon characters and their corresponding photographs were presented on a touch-sensitive computer display. The participant (player *P*) was featured by an additional cartoon representing a pair of animated hands assuming a first-person perspective. Each trial consisted of a single ball exchange depicted by a short animation showing one player handling the ball, and a few seconds later another player catching the ball. If a trial ended with the participant as recipient, he/she became the next trial's sender and had to address the ball to player A, B, or C by touching the corresponding photograph. At game start, probabilities were homogeneous for all players, that is, the participant had a probability $P = 1/3$ of receiving the ball from any of the three players. After a predetermined number of rounds, player profiles diverged such that player A (the "good" profile) sent, on average, 70% of its played balls to the participant (*P*), player B (the "neutral" profile) sent 30% of its played balls to *P*, and player *C* (the "bad" profile) sent 10% of its played balls to *P*. These proportions are represented by the length of the gray arrows in Fig. 1*A*. The game included a monetary incentive to enhance the participant's cognitive engagement in the task. Any player receiving the ball earned 2€ (see *SI Materials and Methods* for details about the algorithm used to dynamically set the three player profiles). To optimize cognitive engagement in the task, the participant was told that each ball received was worth 2 euros, and that when returning the ball two outcomes were possible: either the recipient would toss the ball back to the participant, generating further income, or toss it to another player, earning that player 2 euros. The participant's cumulative gains were displayed on the screen and he/she was led to expect a percentage of the gains at the end of the game. The participant was instructed that the game ended after a total of 80 tosses. The main dependent variable in this experiment was the distribution of the participant's toss choices between players A, B, and C (illustrated for a representative healthy subject by the black arrows in Fig. 1*A*). Also, following completion of the task, the participant estimated, using a subjective seven-point rating scale, their sentiments of "trust" and "preference" with respect to the fictitious players.

Face perception tasks. Pictures of faces were presented on the 17-inch video display of a Tobii 1750 eye tracker and patients' gaze movements were

analyzed offline using ClearView software. Five regions of interest (ROI) on the face were defined: the two eyes, nose, mouth region, forehead, and the two cheeks. A sixth ROI consisted of the portion of the image outside the contour of the face. For each picture, gaze fixation time (in milliseconds) was computed for each of the six ROIs. The number of saccadic eye movements (rapid changes in gaze direction) made for two ROIs, inside and outside the facial contours, was computed (a finer-grained parcellation of the face yields too few eye movement samples) and converted into saccade frequency = (number of saccades)/(total fixation time in ROI) (*SI Materials and Methods*).

Procedure. The study used a randomized, placebo-controlled, double-blind within-subject experimental design. Patients received oxytocin and placebo during two visits to the lab separated by 7 days. They were tested on the social ball-tossing game and on face perception tasks and completed a number of rating scales following the ball game on each visit (Table S5). Healthy subjects were tested on a single visit. General affect was measured after oxytocin and after placebo intake for each participant using the PANAS scale to assess the possible mood-altering effects of oxytocin (*SI Materials and Methods*).

Statistics. Statistical analyses were conducted on the behavioral data in the social ball-tossing game, eye movement measurements, and plasma levels of oxytocin. Nonparametric tests were used because the distribution of the data (number of balls, gaze time, oxytocin levels) was non-Gaussian (*SI Materials and Methods*). Comparisons between groups and drug treatment conditions were made using nonparametric ANOVA (Friedman) as well as Wilcoxon signed-rank and Mann–Whitney rank-sum tests. The sequence effect of treatment was tested using Mann–Whitney rank tests. All tests were evaluated against two-tailed probabilities.

Physiological Measures. ***Oxytocin administration.*** Each subject served as his or her own control and received oxytocin and placebo with a 1-week interval, in a balanced within-subject design. Participants received a single intranasal dose of 24 IU oxytocin (Syntocinon Spray; Novartis; three puffs per nostril, each with 4 IU oxytocin) or placebo 50 min before the start of the experiments (see *SI Materials and Methods* for the details on the measurement of oxytocin).

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