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ORIGINAL ARTICLE

Methylphenidate and desipramine combined treatment improves PTSD symptomatology in a rat model

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Antidepressant medication constitutes the first line pharmacological treatment for posttraumatic stress disorder (PTSD), however, because many patients display no beneficial drug effects it has been suggested that combinations of antidepressants with additional drugs may be necessary. The defining symptoms of PTSD include re-experiencing, avoidance and hyperarousal. In addition, PTSD patients were shown to become easily distracted and often suffer from poor concentration together with indications of comorbidity with attention-deficit hyperactivity disorder (ADHD). Methylphenidate (MPH) is the most common and effective drug treatment for ADHD, thus we aimed to investigate the effects of MPH treatment, by itself or in combination with the antidepressants fluoxetine (FLU) or desipramine (DES). We modified an animal model of PTSD by exposing rats to chronic stress and evaluating the subsequent development of behavioral PTSD-like symptoms, as well as the effects on proinflammatory cytokines, which were implicated in PTSD. We report that while FLU or DES had a beneficial effect on avoidance and hyperarousal symptoms, MPH improved all three symptoms. Moreover, the combination of MPH with DES produced the most dramatic beneficial effects. Serum levels of interleukin-1β (IL-1β) and IL-6 were elevated in the PTSD-like group compared with the control group, and were decreased by MPH, FLU, DES or the combination drug treatments, with the combination of DES+MPH producing the most complete rescue of the inflammatory response. Considering the versatile symptoms of PTSD, our results suggest a new combined treatment for PTSD comprising the antidepressant DES and the psychostimulant MPH.

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INTRODUCTION

Posttraumatic stress disorder (PTSD) is a chronic anxiety disorder that follows an exposure to traumatic events. DSM-V defines PTSD by the coexistence of three clusters of symptoms: re-experiencing, avoidance and hyperarousal, persisting for at least 1 month.[1] Traumatic PTSD-inducing events in adults may be acute or chronic,[2,3] although children and adolescents suffering from PTSD were usually found to be exposed to chronic traumas (physical/sexual abuse).[4,5] Nevertheless, PTSD develops in only a minority of trauma-exposed survivors.[6]

There are a number of suggested PTSD animal models that incorporate various stress paradigms, including exposure to inescapable electric shocks, predator/predator-odor stress or 'single prolonged stress' paradigm (reviewed in Stam[7]). While most models focused on acute stress,[8–10] few implemented continuous and predictable chronic stress.[11,12] Furthermore, timing of the exposure to stress during individuals' developmental trajectory was found to have a crucial role in determining its long-term effects, as we previously reported.[13,14] Similar to humans, there is a marked heterogeneity in the response of animals to stress. However, most studies using PTSD animal models refer to the entire stress-exposed group as a uniform PTSD population, although some reports showed that individual differentiation improved the animal models' face validity.[8,15,16]

The most commonly used medications for PTSD are antidepressants, which relieve symptoms of depression and anxiety. Selective serotonin reuptake inhibitors (for example, fluoxetine) are typically the first line treatment, and are often prescribed interchangeably for the treatment of PTSD. Tricyclic antidepressants (for example, desipramine) or monoamine oxidase inhibitors are generally reserved as second- and third-line strategies due to tolerability issues.[17]

Unfortunately, many PTSD patients fail to adequately respond to the existing pharmacological treatments,[18] with only ~60% patients responding to treatment and approximately 20–30% who achieve full remission.[19] Thus, it seems that the available pharmacotherapies do not offer a sufficient solution for PTSD patients and there is a major need for novel treatment strategies.

Indeed, the heterogeneity of symptom clusters in PTSD as well as the complex psychiatric comorbidities (for example, with depression or substance abuse) further support the notion that combinations of medications may be needed. Therefore, the mainstay of effective treatment for PTSD and its complex psychiatric comorbidities is a combination of treatments (for review see ref. 20).

Human studies suggest that PTSD patients are easily distracted and show poor concentration.[21,22] Indeed, comorbidity between PTSD and attention-deficit/ hyperactivity disorder (ADHD) has been reported.[23,24]

Treatment with the psychostimulant methylphenidate (MPH; Ritalin), a dopamine (DA) and norepinephrine transporters inhibitor, is generally effective in reducing symptoms associated with ADHD.[25–28] However, to our knowledge, only few case

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reports of PTSD patients treated with psychostimulants are available.[29,30]

The excess of inflammatory actions of the immune system in individuals with chronic PTSD was recently suggested.[31] Specifically, increased interleukin-1β (IL-1β), a proinflammatory cytokine, was observed in combat veterans[32] and panic disorder patients.[33] IL-1β was also found to be involved in memory formation and consolidation, thus relevant for the understanding of pathological retention of unpleasant traumatic memories in PTSD.[34,35] Likewise, IL-6 was also found to be increased in the serum[36,37] and cerebrospinal fluid of PTSD patients.[38]

Thus, we aimed to examine the effects of MPH treatment on our modified PTSD animal model, in which we introduced chronic stress in an unpredictable schedule along the pubescence period. Specifically, we tested MPH treatment combined with or without the common (that is, the selective serotonin reuptake inhibitor drug fluoxetine) or the less common (that is, the norepinephrine reuptake inhibitor desipramine) treatments for PTSD-like symptoms. In addition, we examined the possible involvement of IL-1β and IL-6 in the PTSD pathogenesis and MPH treatment.

MATERIALS AND METHODS

Animals

Male Wistar rats were purchased from Harlan (Jerusalem, Israel) at postnatal day (PND) 30 and were housed at the institutional animal facility. Following 5 days of acclimation, rats were randomly assigned to control ($n=20$) or stress ($n=96$) groups. Room temperature was maintained at 23 ± 1 °C with ~67% humidity, on a 12:12 day/night cycle (lights on at 0600 hours) and *ad libitum* food and water access was allowed. All behavioral tests and manipulations were held between 0700 and 1700 hours. This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

All experimental procedures extended over 13 weeks as illustrated in Figure 1a.

Stress procedure and PTSD model

Stress procedure was performed in unpredictable schedule (varying day and hour of exposure), twice a day, (with inter-exposure interval of 1 h), 4 days a week, over 2 weeks during the pubescence period (PND 35–49).

Rats were placed in a fear-conditioning arena with transparent walls and grid floor ('context'). After a 10-s cue (5 Hz, 85 dB clicker tone), rats were exposed to a foot-shock (2 s, 0.8 mA shock) and remained in the chamber for additional 60 s before they were returned to their home cages.

The control rats underwent the same procedure, but without receiving a shock.

PTSD-like animal definition. To mimic the time period between the traumatic event onset and the emergence of PTSD symptoms, 4 weeks following the exposure to stress (PND 77) all rats were tested behaviorally for identification of behavioral measures that depict the core symptoms of PTSD: (I) Re-experiencing was modeled by fear conditioning test. Rats were re-exposed to the cue and/or context of the original stress manipulation and their freezing duration was measured. (II) Hyperarousal was modeled by hypervigilance, as measured in the startle response test. (III) Avoidance was modeled by loss of interest and social withdrawal, expressed in reduced exploration in the open field test and social interaction. The comparison between the control and the stress groups is shown in Supplementary Figure S1.

We utilized the median of each behavioral test as an inclusion border defining PTSD-like rats. However, to reflect the normal distribution characteristics of our data and to avoid an arbitrary criterion such as the median, we further examined the normal distributions of the various behavioral tests. On the basis of the skewness and kurtosis of the various normal distributions, an inclusion border of mean+0.25 s.d. has emerged. The comparison between the stress and the control groups according to these criteria has yielded the same pattern of results, as the median split.

The definition of PTSD-like animals are described schematically in Figure 1b. Only rats that exceeded the mean+0.25 s.d. border, in at least two out of three symptoms (46 out of 96 tested rats) were considered as PTSD-like group. The latter showed significant effects in all symptoms compared with the control group (see Supplementary Figure S2).

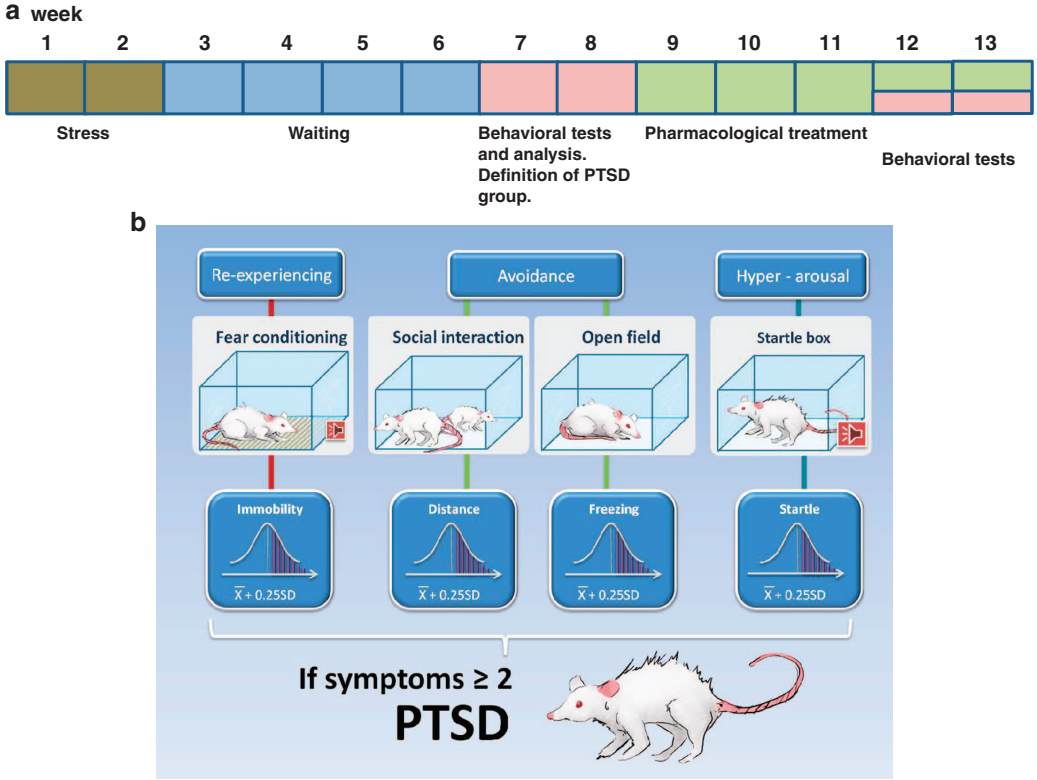

Figure 1. (**a**) Procedures timeline. (**b**) A schematic description of PTSD-like rat definition. PTSD, posttraumatic stress disorder.

Pharmacological treatments

Pharmacological treatments were applied (intraperitoneally) daily (PND 91–127) 9 weeks after the exposure to stress. PTSD-like rats were randomly assigned to the different pharmacological treatment groups; methylphenidate (MPH; $n=6$), fluoxetine (FLU; $n=6$), FLU+MPH ($n=9$), desipramine (DES; $n=7$), DES+MPH ($n=9$) or placebo (injected with saline; $n=9$). Control rats ($n=20$) were injected with saline.

Treatment doses. MPH: 2.7 mg kg^{-1} body weight (Sigma-Aldrich, St Louis, MO, USA);[39] FLU: 10 mg kg^{-1} body weight (Teva Pharmaceutical Industrial Ltd, Petah-Tikva, Israel);[40,41] DES: 10 mg kg^{-1} body weight (Sigma-Aldrich);[41] FLU+MPH or DES+MPH: single injection of the mixture (same doses). All drugs were dissolved in a sterile saline solution. Rats were pharmacologically (as well as saline) treated 1 h before the behavioral testing (PND 112–127), to maintain the pharmacological effects also during the tests period, and to avoid clearance from the central nervous system. The alleged stressful effects of intraperitoneal injection were controlled by the saline-injected group.

Behavioral tests

Post treatment, the behavioral tests below were conducted in the order they appear.

Sucrose preference test

The sucrose preference test (modified from ref. 42) is a two-bottle choice paradigm aimed to evaluate anhedonia. Animals were given free access to two bottles containing water and ascending concentrations of sucrose (0.125, 0.25, 0.5 and 1%) for 1 day per concentration. Intake of water or a sucrose solution was measured by weighing the two bottles before placing them in the cages and after 24 h. To avoid side preference, each day the position of the two bottles in the cage was switched randomly. Sucrose preference was calculated as percentage of sucrose intake from total liquid intake.

Open field test

The open field is made of a black lusterless Perspex box (100 L × 100-W × 40 H, cm). Rats were placed in the corner of the open field (facing the wall). Their behavior (that is, locomotor activity and freezing) was videotaped for 5 min by a CC TV Panasonic camera with post-recording analysis performed using Ethovision XT software (Noldus, Wageningen, The Netherlands).

Social interaction

The social interaction test is conducted in an arena (100 L × 100 W × 40 H, cm) made of black lusterless Perspex. Rats were acclimated to the arena, individually, for 5 min in two subsequent days. On the test day (3rd day), rats from different home cages (but from the same experimental group) were placed for 5 min in the arena and the social interaction was videotaped with post-recording analysis. We measured the 'no interaction' as the time in which the distance between the animals exceeded 50 cm.

Fear conditioning

The fear-conditioning arena (dimension: 45 L × 24 W × 40 H, cm) is made of Plexiglas in different contexts (black or transparent), surrounded with a beam-break frame with an eight lux light. The system is placed in a sound-proof ventilated box (70 L × 40 W × 50 H, cm; Campden, UK). The arena floor consists of 12 grids (6 mm diameter), 12 mm apart. On the first day, in the 'cue condition', rats were placed in a novel context (black arena without grid) then they were introduced to a 10-s cue (5 Hz, 85 dB clicker tone), and their immobility behavior was measured during a 3-min trial. On the next day, in the 'context condition', rats were exposed to the original context (transparent arena with grid) and their immobility behavior was measured during a 3-min trial. In the 'cue+context condition', rats were exposed to the original context (transparent arena with grid) and were introduced to the original 10-s cue (5 Hz, 85 dB clicker tone) followed by a 3-min immobility measurement. The performance was calculated by the Kinder Scientific software (Campden, UK).

Pre-pulse inhibition and startle response

Tests are held in a ventilated sound-proof box (Campden instruments, UK). The test protocol was carried out according to our previous study.[39]

Forced swim test (porsolt test)

Porsolt *et al.*[43] behavioral categories, defined floating as a lack of motion of the whole body while performing only small movements necessary to keep the animal's head above the water. Floating is considered as depression-like behavior.

On the pre-test day, animals were placed individually in a Plexiglas cylinder (transparent acrylic, 60 cm height, 30 cm diameter) filled with water (temperature: 23–25 °C; depth: 40 cm). After 15 min, rats were removed from the water, dried and returned to their home cages. On the test day, rats were placed in the same cylinder for 5 min and videotaped with post-recording analysis.

Cytokines measurement

Twenty-four hours following the behavioral tests, rats were decapitated. Blood samples were centrifuged (2000 *g* at 4 °C for 20 min), serum was collected and stored at − 80 °C until assayed. Serum IL-1β and IL-6 levels were assessed using commercial ELISA kits (R&D Systems, Abingdon, UK) according to the manufacturer's instructions.

Statistical analysis

For each behavioral measure, skewness and kurtosis were calculated to verify normal distribution with similar characteristics, which yielded a mean+0.25 s.d. border for defining PTSD-like rat.

Data were analyzed for statistical significance using two-way analysis of variance (ANOVA) for mixed design, with group as between-subject's factor and fear conditioning conditions/sucrose concentration/PPI pre-intensity, as within-subject's factor. For analyzing differences between two groups we used Student's *t*-test for independent samples. Differences between the various pharmacological treatments tested by one-way ANOVA, followed by *post hoc* Tukey tests. We have calculated a *Z*-score $[(X-\text{mean}X)\times \text{s.d.}^{-1}]$ comprising all behavioral measures (based on mean and s.d. of each measure, in each group) to enable the comparison between different measures that depict PTSD-like symptoms A result was significant when $P<0.05$. All tests were calculated as two-tailed with SPSS V17.0 (Chicago, IL, USA). Results are presented as means ± s.e.m.

RESULTS

Effects of drug treatments on the re-experiencing symptom

In the fear-conditioning test, a significant effect was found for group ($F(6,57)=13.01$, $P<0.0001$) in the context condition (Figure 2a). Saline-injected PTSD-like rats exhibited higher immobility duration compared with the control group. Compared with the saline-injected PTSD-like rats, MPH-treated rats, showed decreased immobility duration. No significant effect was found following FLU treatment, whereas following DES immobility, duration was increased. Surprisingly, the combined treatment of DES+MPH led to a significant decrease in immobility duration. No significant effect was found in both cue and cue+context conditions between the control and the PTSD groups.

Effects of drug treatments on the hyperarousal symptom

In the startle response a significant effect was found ($F(6,57)=22.73$, $P<0.0001$; Figure 2b). Saline-injected PTSD-like rats showed a significantly higher startle response compared with the controls. Compared with the saline-injected PTSD-like rats, MPH-treated rats did not demonstrate any change in the startle response compared with the PTSD group. A significant decrease was found following FLU treatment whereas treatment with both FLU and MPH did not alter the startle response. However, DES treatment (with or without MPH) led to a significantly decreased startle response.

In the pre-pulse inhibition (PPI) test (Figure 2c), a two-way ANOVA for mixed design, with group as between-subject factor

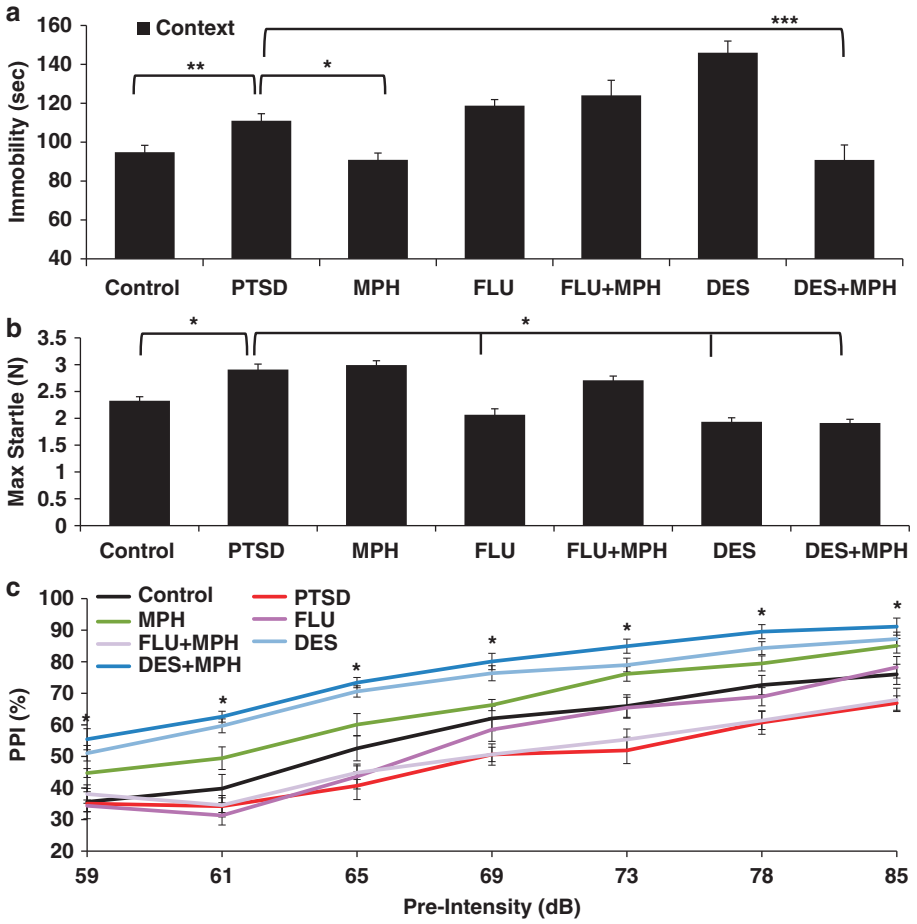

Figure 2. Pharmacological treatments' effect on re-experiencing and hyperarousal symptoms. (**a**) Re-experiencing in fear conditioning test (context condition). MPH or DES+MPH PTSD-treated rats showed lower immobility duration compared with the saline-injected PTSD group; $^{*}P < 0.018$, $^{**}P < 0.013$, $^{***}P < 0.001$. (**b**) Hyperarousal in startle response. FLU, DES or DES+MPH treatments significantly decreased startle response compared with the PTSD group;$^{*}P < 0.0001$. (**c**) Hyperarousal in PPI test. MPH, DES or DES+MPH treatments significantly improved PPI impairment observed in the PTSD group; $^{*}P < 0.012$. Values represent mean ± s.e.m. DES, desipramine; FLU, fluoxetine; MPH, methylphenidate; PPI, pre-pulse inhibition; PTSD, posttraumatic stress disorder.

and pre-intensity as within-subjects, repeated-measures factor, significant effects were found for pre-intensities ($F(6,52) = 114.3$, $P < 0.0001$), group ($F(6,57) = 6.12$, $P < 0.0001$) and group × pre-intensities interaction ($F(36, 302) = 1.63$, $P < 0.016$). Starting at a pre-intensity of 65 dB and onward, PTSD-like rats exhibited lower PPI performance compared with controls (65 dB, 69 dB, 73 dB, 78 dB, 85 dB). MPH-treated rats demonstrated a recovery in PPI performance. Interestingly, although treatment with FLU had no significant effect, treatment with DES (with or without MPH) resulted in the highest PPI performance.

Effects of drug treatments on the avoidance symptom

In the open field test, a significant effect in locomotor activity was found ($F(6,59) = 31.22$, $P < 0.0001$, Figure 3a). Saline-injected PTSD-like rats showed significantly lower locomotor activity compared with the control group. Compared with the saline-injected PTSD-like rats, MPH treatment abolished this decrease (MPH versus PTSD), whereas, surprisingly, FLU, FLU+MPH or DES administration worsened the locomotor activity levels, DES+MPH treatment resulted in a significant recovery of the locomotor activity. A significant effect was also found with respect to freezing duration in the open field test ($F(6,59) = 26.51$, $P < 0.0001$; Figure 3b). Saline-injected PTSD-like rats showed a longer freezing duration compared with the control group. Compared with the saline-injected, PTSD-like rats MPH treatment reduced the freezing duration whereas FLU, FLU+MPH or DES did not. A tendency for improvement was observed in rats treated with DES+MPH. In the social interaction test a significant effect was found ($F(6,38) = 29.52$, $P < 0.0001$; Figure 3c), with saline-injected PTSD-like rats spending more time without interaction compared with the control group. Compared with the saline-injected PTSD-like rats MPH, FLU+MPH, DES and DES+MPH treatments significantly improved social interaction. Moreover, DES+MPH-treated rats showed superior social interaction compared with rats treated with MPH, FLU+MPH and DES.

In the sucrose preference test (Figure 3d), a two-way ANOVA revealed significant effects for sucrose concentration ($F(4,37) = 499.81$, $P < 0.0001$), group ($F(6,40) = 30.15$, $P < 0.0001$) and group × sucrose-concentration interaction ($F(24, 142) = 6.54$, $P < 0.0001$). At sucrose concentrations of 0.5 and 1%, saline-injected PTSD-like rats showed anhedonia compared with the control group, whereas there was no difference in their body weight (data not shown). MPH treatment had no significant effect on sucrose preference. FLU treatment increased the sucrose preference only at 1%, while DES (with or without MPH) rescued sucrose preference in both 0.5 and 1% concentrations.

Measuring total liquid consumption (Figure 3e), significant effects were found for sucrose concentration ($F(4,34) = 45.28$, $P < 0.0001$), group ($F(6,37) = 130.67$, $P < 0.0001$), and group × sucrose concentration interaction ($F(24, 130) = 7.25$, $P < 0.0001$). Saline-injected PTSD-like rats showed significantly lower total

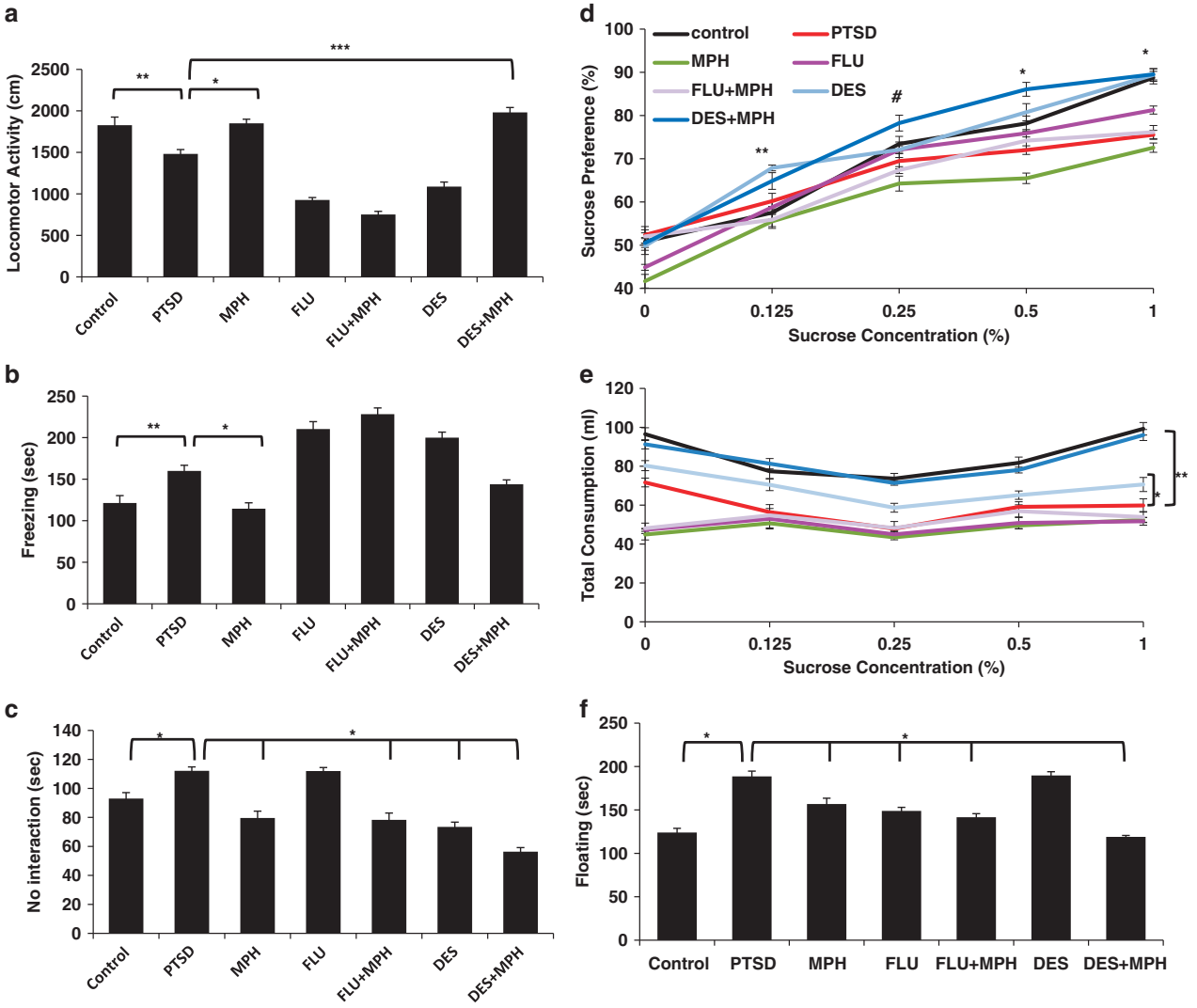

Figure 3. Pharmacological treatments' effect on avoidance symptom. (**a**) Locomotor activity in the OF test was significantly recovered by MPH or DES+MPH treatments, compared with the saline-injected PTSD group; $^{*}P < 0.011$, $^{**}P < 0.002$, $^{***}P < 0.0001$. (**b**) Freezing duration in the OF test was significantly improved by MPH treatment, compared with the PTSD group; $^{*}P < 0.002$, $^{**}P < 0.001$. (**c**) In the social interaction test, MPH, FLU+MPH, DES or DES+MPH treatments significantly improved social interaction compared with the PTSD; $^{*}P < 0.0001$. (**d**) In the SPT, treatment with DES or DES+MPH led to hedonic effect compared with PTSD group in 0.125, 0.5 and 1% concentrations; $^{*}P < 0.0001$, $^{**}P < 0.029$. However, in 0.25% significance was found only for DES+MPH treatment; $^{\#}P < 0.004$. (**e**) Total consumption measures indicated a significant increase following DES and DES+MPH treatments compared with the PTSD group, along all concentrations; $^{*}P < 0.028$, $^{**}P < 0.0001$. (**f**) In the Porsolt test, shorter floating duration was observed following treatments with MPH, FLU, FLU+MPH or DES+MPH compared with PTSD group; $^{*}P < 0.0001$. Values represent mean ± s.e.m. DES, desipramine; FLU, fluoxetine; MPH, methylphenidate; PPI, prepulse inhibition; PTSD, posttraumatic stress disorder; SPT, sucrose preference test.

liquid consumption compared with the control group, along all sucrose concentrations. Compared with the saline-injected PTSD group MPH, FLU and FLU+MPH treatments had no significant effect, whereas treatment with DES (with or without MPH) significantly increased the total consumption, along all concentrations. Surprisingly, DES+MPH completely rescued liquid consumption back to the control level.

Finally, in the Porsolt test (Figure 3f) a significant effect was found in floating duration ($F(6,55) = 29.2$, $P < 0.0001$). Specifically, saline-injected PTSD-like rats significantly spent more time floating compared with the control group. Interestingly, compared with the saline-injected PTSD-like group, a significant decrease in floating duration was observed following all treatments, except DES.

Combined treatment with MPH and DES improved all three symptoms

To compare the overall effectiveness of the various treatments (summarized in Supplementary Table S1), we have calculated a standardized score comprising all behavioral measures (Figure 4). Marked impairment was observed in the saline-injected PTSD-like group ($Z = -1.69$) compared with the control group ($Z = 0$). A slight improvement was observed following FLU ($Z = -1.61$) or FLU +MPH ($Z = -1.48$) treatment, while the MPH or DES treatment yielded a significant improvement ($Z = -0.7$ and $Z = -0.81$, respectively). The combined treatment of DES and MPH showed an additive effect and led to the highest recovery score ($Z = +0.76$).

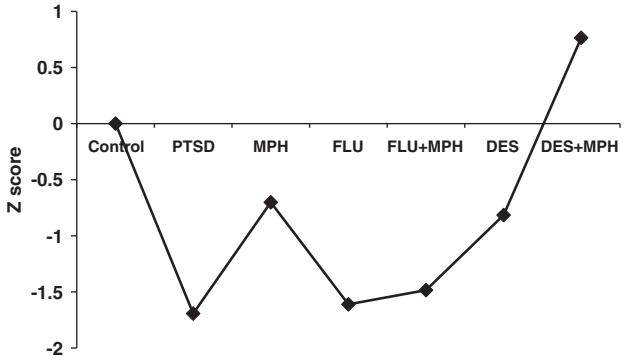

Figure 4. Comparison of the accumulative effect of the various treatments. All measures were standardized relatively to the controls, demonstrating that the saline-injected PTSD group has the lowest *Z*-score that was partially improved by MPH, FLU, FLU +MPH or DES. However, the combined treatment of DES+MPH yielded the uppermost recovery score. DES, desipramine; FLU, fluoxetine; MPH, methylphenidate; PTSD, posttraumatic stress disorder.

Effects of stress and drug treatments on proinflammatory cytokines

For serum level of IL-1β, a one-way ANOVA revealed a significant effect for group ($F(6,38) = 29.77$, $P < 0.0001$; Figure 5a). A significantly higher IL-1β serum level was found in saline-injected PTSD-like rats compared with the controls. All of the drug-treated group displayed significant decreases in IL-1β levels compared with the saline-injected PTSD group, with the MPH treatment producing only a moderate decrease, and the FLU +MPH treatment producing the most dramatic decrease, reducing IL-1β serum concentration to an undetectable level. Both FLU and DES+MPH have recovered IL-1β back to the control level.

For serum level of IL-6, a one-way ANOVA revealed a significant effect for group ($F(6,40) = 16.02$, $P < 0.0001$; Figure 5b). Similarly to the effects on IL-1β, a significantly higher IL-6 serum level was found in saline-treated PTSD-like rats compared with the controls. All treatments (MPH, FLU, FLU+MPH, DES+MPH), excluding DES, significantly decreased IL-6 level compared with the saline-injected PTSD group. The treatment with MPH and DES+MPH was particularly effective, reducing IL-6 serum levels back to the control levels.

DISCUSSION

As PTSD is a complex disorder, which often displays comorbidity with other disorders (for example, depression, alcohol and drug abuse, ADHD), patients may have a great diversity of symptoms.[24,44,45] Given the evidence of PTSD and ADHD comorbidity,[23,24] we treated PTSD-like rats with MPH, combined with or without the antidepressants FLU or DES.

In the re-experiencing symptom, we found that the context served as the most significant stimulus compared with cue and cue+context conditions. Grossberg[46] introduced the stability-plasticity dilemma: the need to keep old memories stable versus the will to maintain enough plasticity to learn new things. This

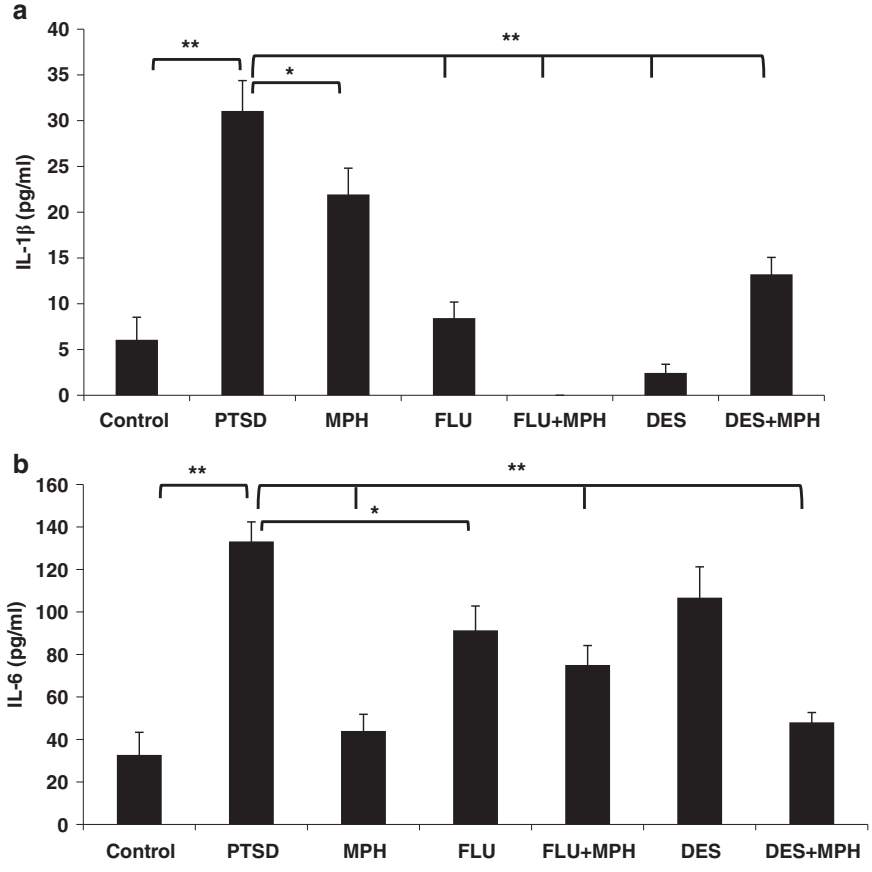

Figure 5. Pharmacological treatments' effect on IL-1β and IL-6 serum concentration. (**a**) Saline-injected PTSD-like rats significantly increase IL-1β level compared with the controls. All treatments significantly decrease IL-1β level compared with the PTSD group; $*P < 0.008$, $**P < 0.0001$. (**b**) PTSD-like rats showed a significantly higher IL-6 serum level compared with the controls. All treatments except for DES significantly decrease IL-6 level compared with the PTSD group; $*P < 0.032$, $**P < 0.0001$. DES, desipramine; FLU, fluoxetine; IL, interleukin; MPH, methylphenidate; PTSD, posttraumatic stress disorder.

preferentiality of the context over cue may reflect the 'stable' versus the 'plasticity' choice. MPH, with or without DES, had a beneficial effect on the re-experiencing symptom. In support of our finding, human studies showed that MPH has a positive impact on emotional processes in adult ADHD patients.[47] In addition, animal studies reported a reduction in immobility in the fear-conditioning test, following MPH administration.[48,49]

In the hyperarousal cluster, we found that FLU, DES or DES +MPH treatments yielded a valuable improvement in the startle response. As the mono treatment with MPH did not result in any change, we assume the effectiveness of the combined treatment of DES+MPH, in this case is due to the anxiolytic effect of DES. In addition, we tested PPI performance, which is conceptualized as a sensorimotor gating mechanism that serves a critical inhibitory action for sensory, cognitive and motor output processing.[50,51] Deficits in PPI were reported in PTSD patients, although they are inconclusive, suggesting abnormalities in information processing mechanisms relevant to sensory or sensorimotor gating.[52] PTSD rats exhibited poor PPI level, maintained long after the exposure to the original stress. We found an improved PPI following all treatments except for FLU+MPH. Superior inhibition was observed in the PTSD-like rats treated with DES or DES+MPH. The beneficial effect of MPH on PPI related tests was previously reported in ADHD patients.[53] However, to the best of our knowledge, we are the first to report an improvement following DES treatment.

In the avoidance cluster we applied various behavioral measures, including activity in the open field, social interaction, sucrose preference and porsolt test. Overall, the combined treatment of DES+MPH led to the most beneficial effect. Particularly, in the open field test, the positive effect may be attributed to MPH which was previously reported to increase rats' activity.[40,54] In the social interaction test, superior performance was found following DES+MPH treatment, presumably attributed to the positive effect of both. Previous studies reported an increase in social interaction following DES administration.[55] We postulate that the observed MPH beneficial effect on social interaction is mediated by its capability to reduce symptoms such as impulsivity[25] and aggression.[56] In the sucrose preference test, the antidepressants FLU or DES led to heighten preference, as expected. Although MPH as a mono treatment did not affect rats' preference, as was previously shown,[57] the combined treatment of DES+MPH resulted in a valuable improvement. In the forced swim test, once again we observed the highly beneficial effect of the combined treatment of DES+MPH, which may be attributed to the effect of MPH. Though DES did not affect floating duration, it facilitated the beneficial effect of MPH.

Research into the underlying neurobiology of PTSD has focused mainly on dysregulation of norepinephrine, serotonin and glutamate. However, accumulated data suggest the relevant role of DA in the pathogenesis of PTSD.[58–60] There is also evidence of genetic alterations in the expression of DA transporter[59] and DA receptor[60,61] in PTSD patients. Moreover, chronic stress has been shown to alter the function of the nucleus accumbens, portions of the prefrontal cortex and the anterior cingulate cortex, which have been associated with the pathophysiology of PTSD.[62,63] Specifically, prefrontal cortex which is involved in problem solving, learning and complex stimulus discriminations has been shown to be less activated in PTSD patients. Anterior cingulate cortex, which is involved in emotional and cognitive components integration, has also been shown to be less activated.[64]

Psychostimulants, by their relative propensity to enhance DA activity in brain regions such as the nucleus accumbens and prefrontal cortex,[65,66] seem to have particular value in targeting the above dysfunctions. However, to our knowledge, only few case reports of PTSD patients treated with psychostimulants are available, all showed a highly beneficial effect.[29,30]

Given the above and the fact that MPH inhibits DA and norepinephrine transporters, the beneficial effects we observed of MPH on the PTSD-like symptoms, may be due to increased DA activity in the nucleus accumbens and prefrontal cortex brain regions. Nevertheless, we were surprised to find superior effects when administrating the combined treatment of DES and MPH. This facilitatory effect of DES, which is known to inhibit norepinephrine reuptake[67] may be explained by the combined pharmacological effect of both treatments, and strengthen by the extensive data on the comorbidity between PTSD and ADHD, which may share a common mechanism.

Apart from the PTSD core symptoms, evidence from human studies indicate an association between PTSD and worsen metabolic profile.[68,69] Specifically, the prevalence rates of metabolic syndrome are 72% in patients with PTSD.[68] Indeed, in our study, though we did not measure directly the entire metabolic status, we found an interesting decrease in total liquid consumption in the PTSD group (that was not accompanied by body weight difference). Once again, a full recovery of liquid consumption was observed following the treatment with DES+MPH.

Current studies also suggest an excess of inflammatory actions of the immune system in individuals with chronic PTSD.[31] Moreover, secretion of cytokines, modulators of the immune response, was shown to correlate with anxiety, depression and impaired memory performance.[70] Specifically, increased IL-1β and IL-6 were observed in PTSD patients compared with control subjects.[32,37] Selective serotonin reuptake inhibitor treatment was shown to significantly reduce IL-1β level,[71] whereas, hydrocortisone administration significantly reduced IL-6 level in PTSD patients.[72] Nevertheless, the effect of PTSD treatment on these cytokines elevation was hardly investigated.

To investigate a possible underlying mechanism to our behavioral findings, we measured the rats' IL-1β and IL-6 serum concentration level. Similar to previous reports,[32,33,36,37] we found a significant increase in serum levels of IL-1β and IL-6 in the PTSD-like group, compared with the controls. To a different degree, all of our examined treatments decreased the cytokines level.

Specifically, while MPH treatment led to a moderate decrease in IL-1β, FLU+MPH or DES treatments led to drastic decrease (lower than the control). Only FLU and DES+MPH recovered IL-1β to control level. Measuring IL-6, DES treatment did not affect the increase observed in the PTSD group. While FLU or FLU+MPH led to a moderate decrease in IL-6, MPH and DES+MPH recovered IL-6 to control level.

Together, the novel suggested dual treatment of DES+MPH seems to exert the most beneficial effect on both IL-1β and IL-6 serum levels, by recovering these cytokines level back to the control group levels.

Previous studies have shown that antidepressants have an anti-inflammatory effect in diverse disorders. Specifically, either FLU or DES treatments has shown to decrease the serum level of IL-1β (refs 73–77) and IL-6.[78–82] To the best of our knowledge, our results are the first to show anti-inflammatory effect of MPH and especially, the effect of the combined treatment of DES+MPH. However, there is a need for more studies to establish the exact mechanisms that are responsible for the immunoregulatory effects of chronic use of both antidepressants and MPH.

To conclude, our results may offer, with the appropriate considerations, a new pharmacological approach for PTSD treatment comprising both the antidepressant desipramine and the psychostimulant methylphenidate. The suggested duo treatment should further be investigated to address open questions regarding the pharmacodynamics and chronicity of the treatment. Yet, our findings may serve as a platform for future human studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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