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OXFORD

REGULAR RESEARCH ARTICLE

IGFBP2 Produces Rapid-Acting and Long-Lasting Effects in Rat Models of Posttraumatic Stress Disorder via a Novel Mechanism Associated with Structural Plasticity

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Abstract

Background: Posttraumatic stress disorder is an anxiety disorder characterized by deficits in the extinction of aversive memories. Insulin-like growth factor 1 (IGF1) is the only growth factor that has shown anxiolytic and antidepressant properties in human clinical trials. In animal studies, insulin-like growth factor binding protein 2 (IGFBP2) shows both IGF1-dependent and IGF1-independent pharmacological effects, and IGFBP2 expression is upregulated by rough-and-tumble play that induces resilience to stress.

Methods: IGFBP2 was evaluated in Porsolt, contextual fear conditioning, and chronic unpredictable stress models of posttraumatic stress disorder. The dependence of IGFBP2 effects on IGF1- and AMPA-receptor activation was tested using selective receptor antagonists. Dendritic spine morphology was measured in the dentate gyrus and the medial prefrontal cortex 24 hours after in vivo dosing.

Results: IGFBP2 was 100 times more potent than IGF1 in the Porsolt test. Unlike IGF1, effects of IGFBP2 were not blocked by the IGF1-receptor antagonist JB1, or by the AMPA-receptor antagonist 2,3-Dioxo-6-nitro-1,2,3,4 tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) in the Porsolt test. IGFBP2 (1 µg/kg) and IGF1 (100 µg/kg i.v.) each facilitated contextual fear extinction and consolidation. Using a chronic unpredictable stress paradigm, IGFBP2 reversed stress-induced effects in the Porsolt, novelty-induced hypophagia, sucrose preference, and ultrasonic vocalization assays. IGFBP2 also increased mature dendritic spine densities in the medial prefrontal cortex and hippocampus 24 hours postdosing.

Conclusions: These data suggest that IGFBP2 has therapeutic-like effects in multiple rat models of posttraumatic stress disorder via a novel IGF1 receptor-independent mechanism. These data also suggest that the long-lasting effects of IGFBP2 may be due to facilitation of structural plasticity at the dendritic spine level. IGFBP2 and mimetics may have therapeutic potential for the treatment of posttraumatic stress disorder.

Significance Statement

IGFBP2 has therapeutic potential for the treatment of PTSD and appears to produces its therapeutic-like effects by facilitating brain plasticity.

Keywords: insulin-like growth factor binding protein 2, insulin-like growth factor I, posttraumatic stress disorder, dendritic spines, resilience.

Introduction

Posttraumatic stress disorder (PTSD) is a major anxiety disorder that is characterized by deficits in the extinction of fearful memories. PTSD affects as many as 30% of individuals that have been directly associated with psychologically traumatic events (Breslau et al., 1998). PTSD is considered a memory disorder in which cues associated with the traumatic event form powerful sensory memories that are resistant to extinction (Parsons and Ressler, 2013). Serotonin specific reuptake inhibitors (SSRIs) are widely used to treat PTSD but with limited efficacy (Ipser et al., 2015), thus there is a great unmet need for the treatment of PTSD.

Growth factor receptor activation has been suggested to have potential for treatment of PTSD (Duman and Monteggia, 2006; Soliman et al., 2010). Insulin-like growth factor I (IGF1) has been approved by the FDA for human use in congenital dwarfism (NDA21-839) and has been reported to have both anxiolytic and antidepressant effects in humans when injected i.p. (Thompson et al., 1998). IGF1 plasma levels are positively correlated with decreased expression of PTSD symptoms in humans (Rusch et al., 2015). In addition, IGF1, brain derived neurotrophic factor, and fibroblast growth factor have all been shown to be decreased in depressed patients and elevated by treatment with conventional antidepressants (Duman and Monteggia, 2006; Sen et al., 2008).

Insulin-like growth factor binding protein 2 (IGFBP2) may have therapeutic potential for the treatment of PTSD. IGFBP2 is the predominant IGF1 binding protein in the brain and cerebrospinal fluid, is present at low levels in the periphery (Ocrant et al., 1990; Poljakovic et al., 2006), and has been shown to cross the blood-brain barrier (Huhtala et al., 2012). IGFBP2 has a molecular weight of 36 kD and has multiple biological effects, some of which are independent of its binding to IGF1, mediated by different binding domains within IGFBP2 (Firth and Baxter, 2002; Azar et al., 2014; Reyer et al., 2015). Rough-and-tumble play robustly induces resilience to stress (Burgdorf et al., 2016) and upregulates IGFBP2 mRNA and protein levels in the medial prefrontal cortex (Burgdorf et al., 2010).

PTSD can be modeled in rodents by measuring emotional responses to stress and extinction of these behaviors. Key symptoms of PTSD in humans are persistent recurrent thoughts of traumatic events, especially when reexposed to cues, and increased anxiety and depressive symptoms (American Psychiatric Association, 2013). In rodents, fear extinction tasks can model the persistence of cue-induced stress responses, whereas repeated stress can model the anxiety and depression symptoms of PTSD (Burgdorf et al., 2015b). In these stress models, increases in negative affect are measured by the Porsolt and novelty-induced hypophagia tests, and decreases in positive affect/anhedonia are measured by the sucrose preference test. In addition, the ultrasonic vocalization test measures both negative (20-kHz ultrasonic vocalization [USVs]) and positive affect (50-kHz USVs).

Chronic unpredictable stress (CUS) can be used to test for rapid-acting therapeutics for PTSD. CUS induces a long-lasting and robust PTSD-like phenotype that models clinical features of PTSD and has been well characterized at the behavioral, physiological, cellular, and molecular levels (Duman and Aghajanian, 2012; Burgdorf et al., 2015b). CUS induces a behavioral phenotype that is responsive to chronic (3 weeks) but not acute or short-term treatment with fluoxetine (Schmidt and Duman, 2007) and is sensitive to rapid-acting therapeutics (Li et al., 2010; Burgdorf et al., 2013). Thus, the CUS model provides a rigorous paradigm for identifying compounds with rapid-acting therapeutic efficacy in PTSD, thereby differentiating IGF1 and IGFBP2 from the currently approved therapeutics for this disorder.

The experiments in this study tested whether IGFBP2 has therapeutic potential for the treatment of PTSD by using a contextual fear conditioning paradigm to measure extinction and consolidation and a CUS paradigm to measure anxiety and depressive-like symptoms of PTSD. IGFBP2 was chosen because of its role in resilience to stress induced by positive affect (Burgdorf et al., 2010). Since the behavioral effects of IGF1 are known to be IGF1 and AMPA receptor dependent, we determined whether the behavioral effects of IGFBP2 were also IGFIR and AMPAR dependent. Finally, we performed dendritic spine analysis in the medial prefrontal cortex and hippocampus 24 hours after dosing to determine whether changes in structural plasticity were associated with the long-lasting behavioral effects of IGFBP2.

Materials and Methods

Animals

Adult male 2- to 3-month-old Sprague-Dawley (SD) rats from Envigo Inc. were used. Rats were housed in Lucite cages with aspen wood chip bedding, maintained on a 12:12 light:dark cycle (lights on at 5 AM), and given ad libitum access to Purina lab chow and tap water throughout the study. All experiments were approved by the Northwestern University Institutional Animal Care and Use Committees.

Drugs

IGFBP2 was purchased from PeproTech, IGF1 was purchased from Shenandoah Biotechnology, the IGF1 antagonist JB1 was purchased from Sigma, and the AMPA/kainate receptor antagonist 2,3-Dioxo-6-nitro-1,2,3,4 tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) disodium salt was purchased from

Abcam. Doses for IGF1 and IGFBP2 were determined by dose response studies (Figure 1), and doses of JB1 and NBQX were chosen from Burgdorf et al. (2015a). All drugs were administered in sterile saline vehicle at a volume of 1 mL/kg.

Porsolt Test

Testing was conducted as described in Burgdorf et al. (2013, 2015a). Animals were placed in a 46-cm-tall x 20-cm-diameter clear glass tube filled to 30 cm with tap water (23 ± 1°C) for 15 minutes on the first day (habituation) and 5 minutes on the subsequent test days at 1 hour postdosing for non-CUS-treated rats, or during the last 5 minutes of the habituation trial for CUS-treated rats 1 hour after dosing or 24 hours after the last rough-and-tumble play session. Water was changed after every other animal. Animals were videotaped. Immobility time was defined as the minimal amount of effort required to keep the animals head above water. Experiments were conducted in a blind manner and scored offline by an experimenter with high inter-rater reliability (Pearson's $r > .9$).

JB1 Studies

The day after Porsolt habituation, non-CUS rats were dosed with IGFBP2 (1 µg/kg i.v.), the IGF1R antagonist JB1 (0.5 mg/kg i.v.), co-administration of both IGFBP2 and JB1, or sterile saline vehicle (1 mL/kg i.v.). All animals received a single 5-minute Porsolt test session 1 hour postdosing (n = 6–8/group).

NBQX Studies

The day after Porsolt habituation, non-CUS-treated rats were dosed with IGFBP2 (1 µg/kg i.v.) or sterile saline vehicle (1 mL/kg i.v.). NBQX (10 mg/kg i.p.) or sterile saline vehicle (1 mL/kg i.p.) was coadministered with IGFBP2 or vehicle. All animals received
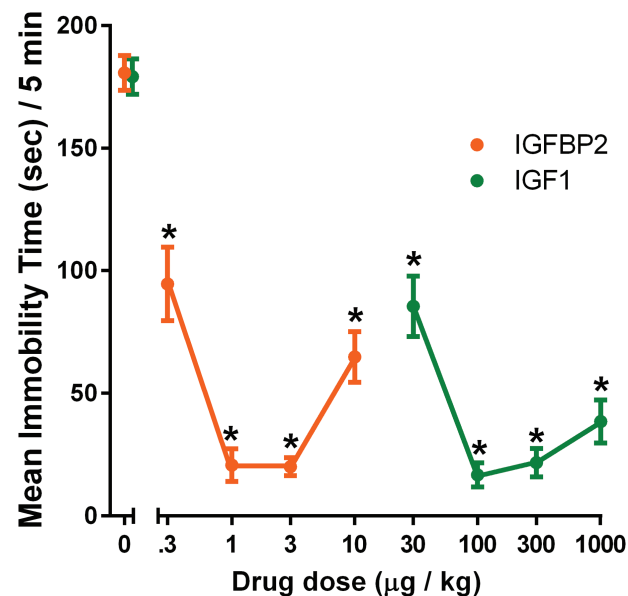
a single 5-minute Porsolt test session 1 hour after dosing (n = 8/group).

Contextual Fear Extinction

Testing was conducted as previously described (Burgdorf et al., 2015b), and the first extinction tests occurred 1 hour postdosing. On the contextual fear training day (D0), animals were placed in a Coulbourn Instruments shock chamber (40 × 40 × 40 cm) for 400 seconds and received three 0.5-mA 1-s footshocks delivered to the floor bars at 90-, 210-, and 330-second timepoints. During extinction, rats were subjected to daily 5-minute nonreinforced (no shock) extinction trials for the first 6 days after training and on day 14 posttraining (consolidation trial). Freezing was quantified via FreezeFrame software (Actimetrics) during the last 3 minutes of each extinction trial. Animals were dosed with a single optimal dose of IGFBP2 (1 µg/kg i.v.), IGF1 (100 µg/kg i.v.), or sterile saline vehicle (1 mL/kg i.v.) 1 hour before the first extinction session (n = 9–11/group).

CUS Procedures

Rats were exposed to a CUS protocol previously shown to elicit depression-like symptoms in rats (Li et al., 2011; Burgdorf et al., 2015a). Animals received 21 days of CUS before dosing and continued to receive CUS until the animals were killed 1 day after the last behavioral test (total of 37 days of CUS). A total of 9 different CUS stressors were used (2 stressors/d). The stressors (days) included rotation on a shaker for 1 hour (3, 9, 13, 19, 24, 28, 33, 37), placement in a 4°C ambient for 1 hour (1, 5, 12, 14, 18, 22, 26, 30, 36), lights off for 3 hours from 10:00 ᴀᴍ to 1:00 ᴘᴍ (2, 10, 17, 23, 31, 34, 37), lights on overnight (1, 5, 8, 13, 16, 22, 32, 34), strobe light overnight (3, 6, 9, 14, 17, 20, 23, 28, 31, 33), 45° tilted cages overnight (4, 7, 11, 15, 18, 21, 25, 29, 35), food and water deprivation overnight (2, 6, 10, 15, 19, 26, 27, 30), crowded housing overnight (4, 7, 11, 16, 21, 25, 29, 35), and isolation housing overnight (8, 12, 20, 24, 27, 32, 36). Animals in the no-CUS group (n = 9) were weighed every 4 days and received behavioral testing without additional stressors. Animals in the CUS groups received a single optimal dose of IGFBP2 (1 µg/kg i.v.; n = 8) or sterile saline vehicle (n = 10).

Sucrose Preference Test

Testing was conducted as described in Li et al., 2011 and Burgdorf et al., 2015a, and testing occurred 3 days postdosing or after the last rough-and-tumble play session. Rats were exposed to a palatable sucrose solution (1%; Sigma) for 48 hours, followed by 4 hours of water deprivation and a 1-hour exposure to 2 identical bottles, one filled with sucrose solution and the other with tap water. Sucrose preference was defined as the ratio of the volume of sucrose vs total volume of sucrose and water consumed during the 1-hour test.

Novelty-Induced Hypophagia (NIH) Test

Testing was conducted as described in Burgdorf et al., 2013, 2015a, and testing occurred 2 days postdosing or after the last rough-and-tumble play session. Animals were food deprived on the night before testing, and lab chow was placed into the center chamber of the open field (40 x 40 x 20 cm) for 10 minutes under dim-red lighting. Between animals, feces and urine were removed from the apparatus. Immediately after NIH testing, the latency to eat in the animal's home cage was determined

Figure 1. A single i.v. dose of insulin-like growth factor binding protein 2 (IGFBP2) produces therapeutic-like effect in the Porsolt test with 100-fold greater potency than insulin-like growth factor I (IGF1). Mean ± SEM immobility time in non-chronic unpredictable stress (CUS)-exposed rats treated with a single injection of IGFBP2 (0.3 to 10 µg/kg i.v.), IGF1 (30–1000 µg/kg i.v.), or saline vehicle (1 mL/kg) and tested 1 hour postdosing. *P < .05 Fisher's PLSD posthoc test vs vehicle. n = 7 to 8/group. Data for IGF1 was adapted from Burgdorf et al. (2015a).

as a control. Animals were videotaped and latency (in seconds) for the animal to take the first bite of food, as well as locomotor activity (in line crosses), was scored offline by an experimenter blinded to the treatment condition.

USV Test

Heterospecific rough-and-tumble play was conducted as previously described (Burgdorf et al., 2013, 2015a), and testing occurred 3 hours and postdosing or 1 day after the last rough-and-tumble play session. Heterospecific rough-and-tumble play stimulation was administered by the experimenter's right hand. The experimenter was blind to the treatment condition of the animals. Animals received 3 minutes of heterospecific rough-and-tumble play consisting of alternating 15-second blocks of heterospecific play and 15 seconds of no-stimulation. High-frequency USVs were recorded and analyzed by sonogram in a blind manner as described previously (Burgdorf et al., 2011). Animals were not habituated to play stimulation before dosing and testing. Using this paradigm, we have shown that the increase in 50-kHz USVs that occurs across trial blocks reflects positive emotional learning (Burgdorf et al., 2011).

Dendritic Spine Morphology Analysis

Dendritic spine analyses were conducted as described in Burgdorf et al., 2015c. Animals were dosed with a single dose of IGFBP2 (1 µg/kg i.v.), IGF1 (100 µg/kg i.v.), or 0.9% sterile saline vehicle (1 mL/kg), and 24 hours postdosing they were deeply anesthetized (isoflurane) and brains fixed via cardiac perfusion using 4% paraformaldehyde. Brains were stored in ice cold 0.1 M phosphate buffer and stored at 4°C until sectioning. Brains were sectioned using a tissue Vibratome (Leica VT1000) to collect sections (300 µm thick) from the anterior to posterior extremes of each brain. Ballistic dye labeling (DiI and DiO; 3 mg dissolved in methylene chloride and coated on tungsten particles) was performed using a commercially available gene gun (Bio-Rad, Hercules, CA) to label target neurons. Thick sections were mounted to slides with raised barriers using ProLong Gold (Life Technologies, Gaithersburg, MD) and cover slipped. Laser-scanning confocal microscopy (Olympus FV1000) was performed using a 63× objective (1.42 NA) to scan individually labeled neurons at high resolution (0.103- × 0.103- × 0.33-µm voxels). Target neurons were identified in the brain regions of interest by anatomical location and cell morphology. Microscopy was performed blind to experimental conditions. A minimum of 5 cells per animal were sampled from each of the 2 regions. For medial prefrontal cortex (MPFC), samples (50 µm) were collected from proximal tufts. Two positions from dentate granule neurons in the hippocampus were selected based on well-described discriminate connectivity between these laminae (Llorens-Martín et al. 2014): (1) primary dendrites within the inner molecular layer, and (2) secondary dendrites from the middle molecular layer. For dentate granule neurons, samples (50 µm) were collected from primary dendrites starting at 100 µm from the soma or the secondary dendrites 50 µm from the branch point.

Blind deconvolution (AutoQuant) was applied to raw 3-dimensional digital images that were then analyzed for spine density and morphology by trained analysts. Individual spines were measured manually for head diameter, spine length, and spine neck diameter from image Z-stacks using software custom-designed by Afraxis Inc. Each dendrite was analyzed by 3 to 4 independent analysts. Automated image assignment software

(C++) distributed images to analysts in a randomized manner and ensured that each analyst performed measurements of near equal numbers of dendrites per group. Analysts were blinded to all experimental conditions (including treatment, brain region, and cell type). Statistical analysis of interanalyst variability for each dendrite was examined online and used to eliminate dendrites that did not meet interanalyst reliability criteria. For spine density and spine morphological classification, data across analysts were averaged to report data for each dendrite. Identification, measurement, and classification of dendritic spines were performed as previously described (Ota et al., 2014). For the purposes of the current study, we have denoted thin spines as "immature" to underscore their vulnerability to modification and role in recent encoding processes relative to more "mature" classes (i.e., mushroom and stubby classes), which are more closely associated with long-term memory storage (Young et al., 2014). Data population values (n's) were reported for dendrites collected equally from all rats.

Statistical Analysis

Data were analyzed by ANOVA, followed by Fisher's PLSD post-hoc test (Statview). The level of statistical significance was set at $P < .05$.

Results

IGFBP2 Is 100 Times More Potent Than IGF1 in the Porsolt Test and Is IGF1- and AMPA-Receptor Independent

IGFBP2 (0.3 to 10 µg/kg i.v.) reduced immobility time in the Porsolt test 1 hour postdosing [$F(4, 31) = 51.99$, $P < .05$; Fisher's PLSD posthoc test all IGFBP2 doses vs vehicle] (Figure 1). The lowest maximally effective dose of IGFBP2 was 1 µg/kg i.v. [Fishers PLSD posthoc test 0.3 vs 10, $P < .05$; 0.3 vs all other IGFBP2 doses, $P < .001$]. However, unlike IGF1, the antidepressant-like effect of IGFBP2 was not blocked by coadministration of either JB1 or NBQX [$F(3,32) = 129.17$, $P < .0001$; Fisher's PLSD posthoc test for vehicle vs all other treatments] (Figure 2).

A Single Dose of IGFBP2 or IGF1 Facilitated Contextual Fear Extinction and Consolidation

IGFBP2 (1 µg/kg iv) or IGF1 (100 µg/kg iv) facilitated contextual fear extinction as measured by reduced freezing time [$F(2, 28) = 8.80$, $P < .05$; Fisher's PLSD posthoc test IGFBP2 or IGF1 vs vehicle from repeated-measures ANOVA analysis; $P < .05$; Fisher's PLSD posthoc test IGFBP2 vs vehicle for D1-6 and D14, and IGF1 vs vehicle for D2-6 and D14 Fisher's PLSD posthoc test on individual test days] (Figure 3A). IGFBP2 or IGF1 also facilitated contextual fear consolidation as measured by the change in freezing score from the last extinction day (D6) compared with the consolidation day 14 [$F(2, 28) = 5.99$, $P < .05$; Fisher's PLSD posthoc test IGFBP2 or IGF1 vs vehicle] (Figure 3B).

A Single i.v. Dose of IGFBP2 Produces a Rapid-Acting and Long-Lasting Therapeutic-Like Effect in a CUS Model of PTSD

IGFBP2 (1 µg/kg, i.v.) treatment in rats exposed to CUS significantly reduced immobility time in the Porsolt test (1 hour postdosing) compared with CUS-treated vehicle rats, and CUS vehicle rats showed increased immobility times compared with
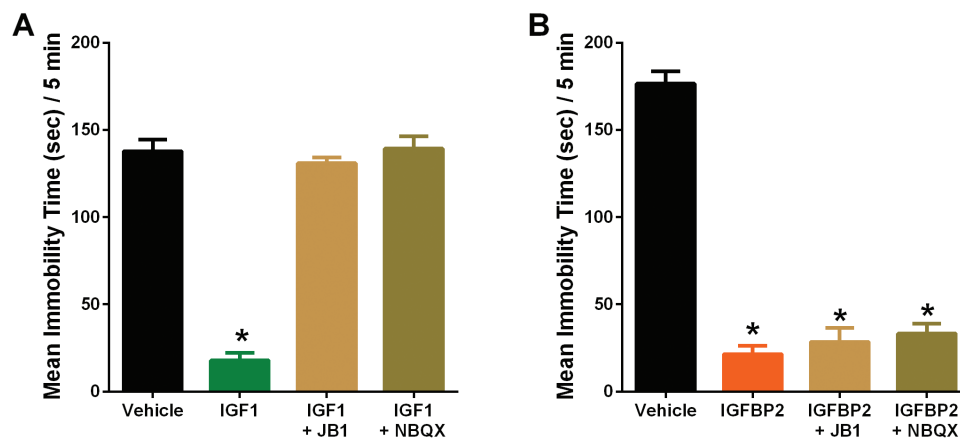
Figure 2. The therapeutic-like effects of insulin-like growth factor I (IGF1), but not insulin-like growth factor binding protein 2 (IGFBP2), are IGF1R- and AMPA-receptor (AMPAR) dependent. (A) Mean ± SEM immobility time in non-chronic unpredictable stress (CUS)-exposed rats treated with a single i.v. injection of IGF1 (100 μg/kg), coadministration of IGF-1 and JB1 (0.5 mg/kg i.v.) or NBQX (10 mg/kg i.p.), or saline vehicle (1 mL/kg), and tested 1 hour postdosing. (B) Mean ± SEM immobility time in non-CUS-exposed rats treated with a single i.v. injection of IGFBP2 (1 μg/kg i.v.), coadministration of IGFBP2 and JB1 (0.5 mg/kg, i.v.), or 2,3-Dioxo-6-nitro-1,2,3,4 tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) (10 mg/kg, i.p.), or saline vehicle (1 mL/kg) and tested 1 hour postdosing. *$P < .05$ Fisher's PLSD posthoc test vs vehicle. n = 6 to 9/group. Data for IGF1 were adapted from Burgdorf et al. (2015a).
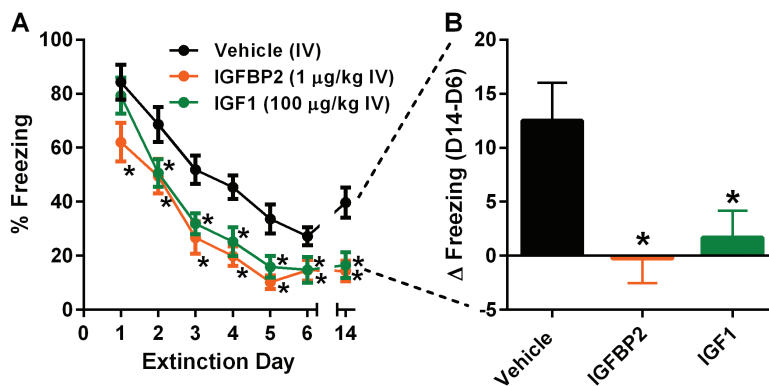

Figure 3. A single dose of insulin-like growth factor binding protein 2 (IGFBP2) or insulin-like growth factor I (IGF1) facilitated contextual fear extinction and consolidation. Mean ± SEM percent time freezing in animals dosed with IGFBP2 (1 μg/kg i.v.), IGF1 (100 μg/kg i.v.), or sterile saline vehicle (1 mL/kg i.v.) 1 hour before extinction day 1. Three shocks (0.5 mA, 1 s) were administered 90, 210, and 330 s after animals were placed in the conditioning chamber on D0. (A) During extinction, rats were submitted to a 5-minute nonreinforced test trial every 24 hours for 6 consecutive days (D1-6), and on day 14 postconditioning (the consolidation trial) and freezing was quantified via FreezeView software and was measured during the last 3 minutes of each trial. (B) Consolidation was measured by the increase in freezing behavior on the last extinction day compared with the consolidation day (D14-D6). n = 9–11 rats/group. *$P < .05$ Fisher's PLSD posthoc test vs vehicle.

no-CUS-treated rats [$F_{(2, 21)} = 63.61$, $P < .0001$; Fisher's PLSD posthoc test IGFBP2 vs vehicle, or vehicle vs no-CUS control, $P < .0001$] (Figure 4A). Furthermore, IGFBP2 increased sucrose preference scores in CUS-treated rats (4 days postdosing) compared with CUS-treated vehicle rats, while CUS vehicle-treated rats showed significantly decreased sucrose preference scores compared with no-CUS-treated rats [$F_{(2, 21)} = 14.3$, $P = .0001$; Fisher's PLSD posthoc test IGFBP2 vs vehicle, $P < .0001$; vehicle vs no-CUS control, $P < .01$] (Figure 4B).

IGFBP2 also significantly decreased feeding latency in a novel environment in the NIH test (2 days postdosing) compared with CUS-treated vehicle rats, while CUS vehicle rats showed longer feeding latencies compared with no-CUS-treated rats [$F_{(2, 21)} = 7.78$, $P < .01$; Fisher's PLSD posthoc test IGFBP2 vs vehicle, vehicle vs no-CUS control, $P < .01$], while no changes were seen in latency to eat in the homecage [$F_{(2, 26)} = 0.4$, $P > .05$; data not shown] or line crosses in the novel environment [$F_{(2, 26)} = 0.3$, $P > .05$; data not shown] (Figure 4C).

Finally, IGFBP2 increased weight gain (1 week postdosing) compared with CUS-treated vehicle rats, while CUS vehicle rats showed decreased weight gain compared with no-CUS-treated

rats [$F_{(2, 21)} = 7.78$, $P < .01$; Fisher's PLSD posthoc test IGFBP2 vs vehicle, vehicle vs no-CUS control, $P < .01$] (Figure 4D).

IGFBP2 Reversed CUS-Induced Deficits in Positive Emotional Learning

IGFBP2 in CUS-treated rats increased rates of hedonic 50-kHz USVs in response to a temporal CS that predicted heterospecific play (positive emotional learning) compared with vehicle CUS-treated rats, and CUS vehicle rats showed decreased CS-elicited hedonic 50-kHz USVs compared with no-CUS-treated rats [$F_{(2, 21)} = 7.19$, $P < .01$; Fisher's PLSD posthoc test IGFBP2 vs vehicle, vehicle vs no-CUS control, $P < .01$] (Figure 4E). IGFBP2 in CUS-treated rats increased rates of hedonic USVs (Figure 4F) and reduced rates of aversive 20 kHz (Figure 4G) compared with vehicle CUS-treated rats, while CUS vehicle-treated rats showed decreased hedonic 50-kHz USVs and increased aversive 20-kHz USVs compared with no-CUS-treated rats [hedonic USVs $F_{(2, 21)} = 58.59$, $P < .0001$; Fisher's PLSD posthoc test IGFBP2 vs vehicle, vehicle vs no-CUS control, $P < .0001$; aversive USVs $F_{(2, 21)} = 8.29$, $P < .01$ Fisher's PLSD posthoc test IGFBP2 vs vehicle, vehicle vs
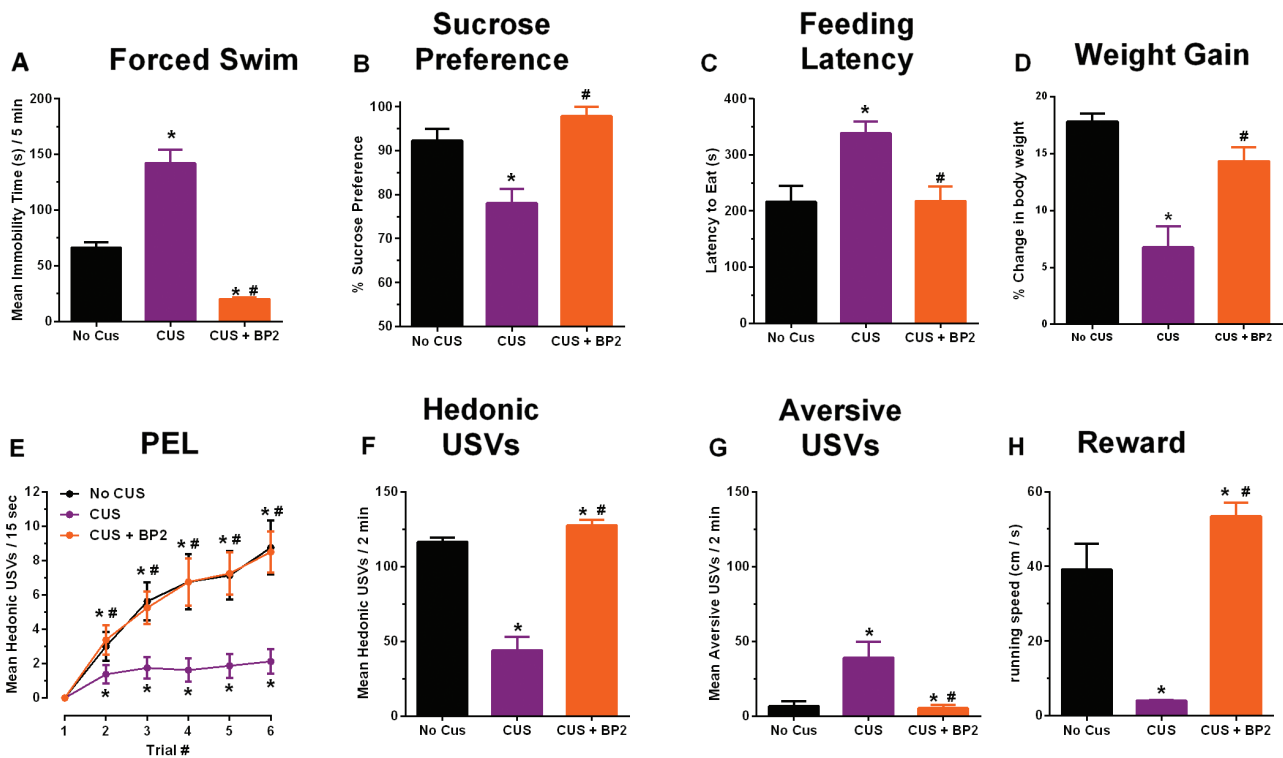
Figure 4. Insulin-like growth factor binding protein 2 (IGFBP2) produces therapeutic-like effects in a chronic unpredictable stress (CUS) model of posttraumatic stress disorder (PTSD). (A) Mean ± SEM immobility time in the Porsolt forced swim test (FS) in naïve (n = 8) and CUS-exposed (n = 8) rats, and CUS-exposed (n = 8) rats that were pretreated with IGFBP2. (B) Mean ± SEM sucrose preference (SP) in the sucrose preference test. (C) Mean ± SEM latency to feed in the novelty induced hypophagia (NIH) test. (D) Mean ± SEM change in body weight (compared with CUS day 0). (E) Mean ± SEM hedonic 50-kHz ultrasonic vocalizations (USVs) in response to a conditioned stimuli that predicts heterospecific play. Mean ± SEM hedonic 50-kHz (F) and aversive 20-kHz (G) USVs. (H) Mean ± SEM running speed to self-administer heterospecific play. n = 8/group. *P < .05 Fisher's PLSD posthoc test vs no-CUS group; #P < .05 Fisher's PLSD posthoc test vs CUS group.

no-CUS control, P < .01]. As shown in Figure 4H, IGFBP2 in CUS-treated rats also significantly increased running speed to self-administer heterospecific play vehicle CUS-treated rats, while CUS vehicle rats showed decreased running speed compared with no-CUS-treated rats [F(2, 26) = 32.72, P < .0001; Fisher's PLSD posthoc test IGFBP2 vs vehicle, vehicle vs no-CUS control, P < .0001].

In No-CUS Rats, IGFBP2 Was Active in the Porsolt, Sucrose Preference, NIH, and Positive Emotional Learning Tests, but Did Not Alter Body Weight

In no-CUS rats, IGFBP2 (n = 9) compared with vehicle (n = 9) reduced immobility time in the Porsolt test [F(1, 16) = 40.1, P < .0001; supplemental Figure 2A], increased sucrose preference [F(1, 16) = 6.2, P < .05; supplemental Figure 2B], decreased feeding latency in the NIH test [F(1, 16) = 19.0, P < .001; supplemental Figure 2C], increased positive emotional learning during the final 2 testing blocks [F(1, 16) = 10.5, P < .01; supplemental Figure 2E], increased total hedonic USVs [F(1, 16) = 59.0, P < .0001; supplemental Figure 2F], reduced total aversive USVs [F(1, 16) = 12.0, P < .001; supplemental Figure 2G], and increased the rewarding value of play as indexed by running speed [F(1, 16) = 6.9, P < .05; supplemental Figure 1H] but did not alter body weight gain 7 days postdosing [F(1, 16) = 1.6, P > .05; supplemental Figure 1D]. Although the vehicle non-CUS values appear to be closer to the vehicle CUS values of the previous experiment, they were within the range of non-CUS control values based on previous studies using these assays (Burgdorf et al., 2009, 2011, 2013).

IGFBP2 Increased Mature Spine Density in MPFC Layer V Pyramidal Neurons, and Both IGFBP2 and IGF1 Increased the Density of Mature Spines and Total Spines in Dentate Granule Neurons of the Hippocampus

IGFBP2 increased the density of mature mushroom spines in MPFC layer V pyramidal neuron proximal tufts [F(2, 77) = 3.4, P < .05; Fisher's PLSD posthoc test IGFBP2 vs vehicle] but did not alter the density of other spine types or the total number of spines in this region (all P > .05) (Figure 5A). IGF1 increased the density of mature stubby spines and the total number of spines in the inner molecular layer of the dentate gyrus [stubby spines - F(2, 71) = 3.7, P < .05; Fisher's PLSD posthoc test IGF1 vs vehicle; total spines - F(2, 71) = 3.9, P < .05; Fisher's PLSD posthoc test IGF1 vs vehicle], while the other spine types did not change (all Ps > 0.05) (Figure 5B). Lastly, IGFBP2 increased the density of mature stubby spines and the total number of spines in the middle molecular layer of the dentate gyrus [stubby spines - F(2, 70) = 3.3, P < .05; Fisher's PLSD posthoc test IGFBP2 vs vehicle; total spines - F(2, 71) = 3.5, P < .05; Fisher's PLSD posthoc test IGFBP2 vs vehicle], while the other spine types did not change (all P > .05) (Figure 5C).

Discussion

The data presented here demonstrate that a single dose of IGFBP2 (1 µg/kg i.v.) can induce rapid- and long-lasting effects in multiple rat models of PTSD. These effects of IGFBP2 were IGF1 and AMPA receptor independent. The effects of IGFBP2 may not be due to direct interactions with CUS-induced mechanisms
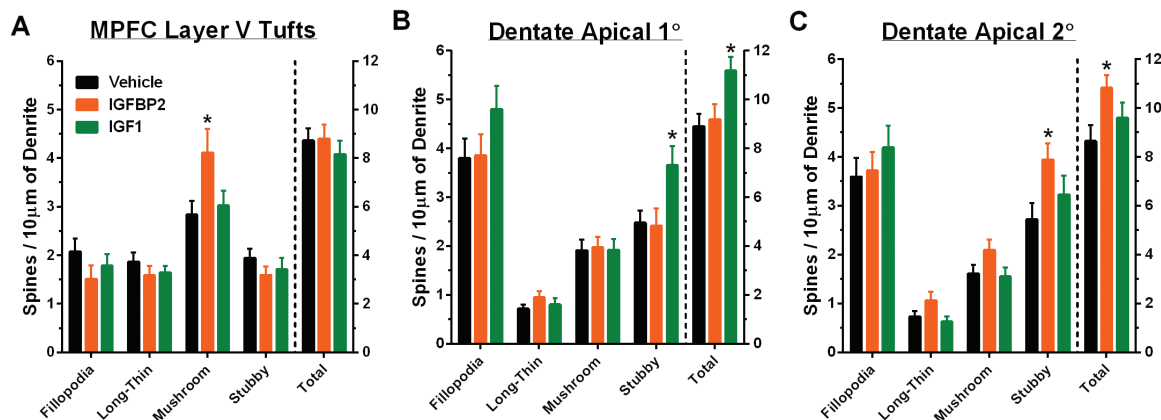
Figure 5. Insulin-like growth factor binding protein 2 (IGFBP2) (1 µg/kg i.v.) and insulin-like growth factor I (IGF1) (100 µg/kg i.v.) differentially increased the density of mature dendritic spines and total spine density in either the MPFC or hippocampus 24 hours postdosing. In male 2- to 3-month-old rats. (A) IGFBP2 (1 µg/kg i.v.) significantly increased the density of mature mushroom spines in MPFC layer V tufts; (B) IGF1 (100 µg/kg i.v) increased the density of mature stubby spines as well as total spine density in the primary apical dentate gyrus (100–150 µM from the dendrite). (C) IGFBP2 increased the density of mature stubby spines as well as total spine density in the secondary apical dentate gyrus (50 µM from the dendritic branch). Mean ± SEM. n = 17 to 31 cells/group (n = 3–4 rats/group; 716–1333 spines/group), *$P <$ .05 Fisher's PLSD posthoc test vs vehicle.

leading to pathophysiology, since the same drug effects were seen in non-CUS rats (supplemental Figure 2). However, given that each of the non-CUS models used in the present studies experienced a preexposure stress (iv injection), IGFBP2 may reverse the effects of both acute and chronic stress. Both fluoxetine and ketamine are clinically effective treatments for PTSD (Connor et al., 1999; Feder and Murrough, 2015) and in preclinical models can reverse the effects of both acute stress and CUS (Cryan et al., 2005; Willner, 2005; Li et al., 2010, 2011). The behavioral effects of IGFBP2 were also associated with increased structural plasticity as measured by mature dendritic spine density in the medial prefrontal cortex and hippocampus. These data suggest that the therapeutic effects of IGFBP2 are mediated by a novel pharmacological pathway.

Both IGFBP2 and IGF1 show unique rapid-acting and long-lasting therapeutic-like effects in multiple models of PTSD. SSRIs are typically used to treat PTSD but have limited efficacy, require 3 to 4 weeks of daily dosing to show maximal efficacy, and are not as effective in treating anhedonia symptoms (Knutson et al., 1998; Ipser et al., 2015). Recently, ketamine has been shown to have rapid-acting therapeutic effects in PTSD (Feder and Murrough, 2015); however, the addictive and psychotomimetic properties of ketamine limits its widespread therapeutic use. Exposure therapy is an extinction-based psychotherapy that is widely used for the treatment of PTSD (Bisson et al., 2013); however, the therapeutic effects wane after treatment has terminated (Tarrier and Sommerfield, 2004), thus demonstrating poor consolidation. In contrast, the therapeutic-like effects of IGFBP2 and IGF1 occur as early as 1 hour and last at least 2 weeks after a single dose and show efficacy in the learning paradigms, as well as in the positive and negative affective symptoms of PTSD. Therapeutic doses of IGFBP2 and IGF1 are not associated with psychotomimetic side effects such as changes in locomotor activity. Lastly, the therapeutic-like effects of IGFBP2 and IGF1 are maintained during the consolidation period after extinction training has ended.

These data suggests that IGFBP2 induces its therapeutic-like effects in PTSD via a novel mechanism. Although IGFBP2 is an IGF1 chaperone, it has also been shown to have IGF1-independent effects via interaction with non-IGF1 binding domains (Reyer et al., 2015). The effects of IGF1 are due to activation of the IGF1 receptor, followed by downstream AMPA

receptor activation (Burgdorf et al., 2015a). In contrast to IGF1, the therapeutic-like effects of IGFBP2 in the Porsolt test were not blocked by either an IGF1 or AMPA receptor antagonist. It is likely that NBQX or JB1 would not block the effects of IGFBP2 in fear extinction/consolidation given that the mechanism of action of IGFBP2 may be shared across PTSD-related behaviors. In addition, IGFBP2 was 100 times more potent than IGF1 in the PTSD models used. IGFBP2 is a relatively large circulating protein (36 kD) with multiple binding domains, and it appears likely that the behavioral effects of IGFBP2 are due to interaction with a novel target via a non-IGF1 binding domain.

Structural plasticity may underlie the long-lasting effects of IGFBP2 and IGF1. Fluoxetine and ketamine have been shown to increase mature dendritic spine density in the hippocampus and/or medial prefrontal cortex. While ketamine increases immature spines, mature spines, and total number of spines (Li et al., 2010, 2011), chronic fluoxetine treatment increases both the density and proportion of mature spines without affecting total spine number (Ampuero et al., 2010). In contrast, IGFBP2 and IGF1 show a third pattern of spine alterations: an increase in only mature dendritic spines, with a concomitant increase in total spine number. The increase in structural plasticity induced by IGF1 and ketamine is also associated with an enhancement in metaplasticity, that is, a lowered threshold for plastic changes in synapse strength, measured as an increased magnitude of LTP induced in slices 24 hours postdosing (Burgdorf et al., 2013, 2015b, 2015c; Graef et al., 2015). In addition, the effects of IGF1 and IGFBP2 are region dependent and are mainly seen in the MPFC, a structure widely implicated in PTSD/anxiety disorders (Shin and Liberzon, 2010). Future studies should examine the effect of IGFBP2 on LTP, metaplasticity, and dendritic spine dynamics in CUS-treated rats to evaluate the therapeutic potential of IGFBP2.

The findings reported here suggest that IGFBP2 may have therapeutic value for the treatment of PTSD, and that IGFBP2 produces these effects via a novel mechanism of action. Future studies will be needed to determine which binding domain(s) of IGFBP2 are responsible for its therapeutic-like effects, and the site(s) of action responsible for these effects. In addition, other IGFBPs should be examined given that some of them have been shown to have protective effects in stress as well as hypoxia-ischemia, which could be relevant to PTSD (Guan, 2011;

[Basta-Kaim et al., 2014](#)). Thus, IGFBP2 and mimetics offer the potential for a new generation of therapeutics for PTSD.

Supplementary Material

Supplementary data are available at *International Journal of Neuropsychopharmacology* online.

Statement of Interest

Acknowledgments

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