UNCOVERING THE MOLECULAR BASIS OF POSITIVE AFFECT USING ROUGH-AND-TUMBLE PLAY IN RATS: A ROLE FOR INSULIN-LIKE GROWTH FACTOR I

J. BURGDORF,* R. A. KROES,* M. C. BEINFELD,† J. PANKSEPP,‡ and J. R. MOSKAL*†

*Falk Center for Molecular Therapeutics, Department of Biomedical Engineering, McCormick School of Engineering and Applied Sciences, Northwestern University, Evanston, IL 60201, USA
‡Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine, Boston, MA 02111, USA
†Department of Veterinary Comparative Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine, Washington State University, Pullman, WA 99163, USA

Abstract—Positive emotional states have been shown to confer resilience to depression and anxiety in humans, but the molecular mechanisms underlying these effects have not yet been elucidated. In laboratory rats, positive emotional states can be measured by 50-kHz ultrasonic vocalizations (hedonic USVs), which are maximally elicited by juvenile rough-and-tumble play behavior. Using a focused microarray platform, insulin-like growth factor I (IGFI) extracellular signaling genes were found to be upregulated by hedonic rough-and-tumble play but not depressogenic social defeat. Administration of IGFI into the lateral ventricle increased rates of hedonic USVs in an IGFI receptor (IGFIR)-dependent manner. Lateral ventricle infusions of an siRNA specific to the IGFIR decreased rates of hedonic 50-kHz USVs. These results show that IGFI plays a functional role in the generation of positive affective states and that IGFIR-dependent signaling is a potential therapeutic target for the treatment of depression and anxiety. © 2010 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: emotion, insulin-like growth factor I, ultrasonic vocalizations.

Positive affect induces resilience to depression and is an under-examined area for development of novel therapeutics. Positive affective states, as studied longitudinally in humans, confer resilience to depression and anxiety, and lead to an increase in overall health and a decrease in mortality from all causes (Lyubomirsky et al., 2005). Psychosocial interventions that increase positive affective states have been shown to reduce levels of depression and anxiety (Lee Duckworth et al., 2005). Currently available antidepressants do not adequately treat the symptoms of decreased positive affect associated with depression (Nierenberg et al., 1999; Nutt et al., 2007), and less than 30% of depressed patients achieve remission with first-line antidepressants (Trivedi et al., 2006). The molecular mechanisms underlying positive affective states are poorly understood and require efficient animal models.

Rat 50-kHz ultrasonic vocalizations (hedonic USVs) is a validated model for the study of positive affective states. Human positive affective states and rat hedonic USVs are elicited by the same stimuli and have homologous neuroanatomical and neurochemical underpinnings (Burgdorf and Panksepp, 2006). In humans, the most robust elicitor of positive affective states is positive social interaction (Stone et al., 2006). In rats, rewarding social interactions (i.e., mating and rough-and-tumble play) uniformly increase rates of hedonic USVs (Knuston et al., 1998; Burgdorf and Panksepp, 2006; Burgdorf et al., 2008), whereas aversive stimuli such as social defeat, frustrating non-reward, an illness-inducing dose of lithium chloride, and foot shock all decrease levels of hedonic USVs (Burgdorf et al., 2000, 2001b; Burgdorf and Panksepp, 2006). The rewarding value of social, drug, and electrical brain stimulation is positively correlated with rates of hedonic USVs (Burgdorf et al., 2001a, 2007; Burgdorf and Panksepp, 2006). Amphetamine, opiates, and direct electrical brain stimulation of the accumbens elicit both positive affect in humans and hedonic USVs in rats (Zacny et al., 1994; Burgdorf et al., 2001a, 2007; Drevets et al., 2001; Okun et al., 2004; Burgdorf and Panksepp, 2006; Boecker et al., 2008). In addition, alternative non-hedonic interpretations of hedonic USVs (i.e., non-valenced arousal, non-hedonic appetitive behavior, or non-hedonic social contact) are not supported by the available data (Burgdorf and Panksepp, 2006; Burgdorf et al., 2008).

The frontal and parietal cortices have been identified as key regulators of positive affective states. In humans, multiple types of positive affective stimuli have been shown to increase neuronal activity in the frontal cortices (Damasio et al., 2000; Blood and Zatorre, 2001; Kringelbach et al., 2003; Nitschke et al., 2004). In non-human primates, electrophysiological studies show that orbital frontal neurons are the most specifically tuned to the hedonic quality of tastants (Rolls, 2008). In laboratory rats, electrical stimulation of the medial frontal cortex robustly elicits hedonic USVs and supports self stimulation (Burg-
dor et al., 2007). In contrast, selective lesions of the parietal cortex markedly reduce rough-and-tumble play behavior, which has been shown to be the most robust natural elicitor of hedonic USVs (Panksepp et al., 1994).

To further elucidate the molecular mechanisms of positive affect, we used an in-house-fabricated, focused microarray platform that, when coupled with appropriate bioinformatics tools, provided a systematic approach for identifying significant gene families associated with positive emotional states (Kroes et al., 2006, 2007). Using this approach, the Insulin-Like growth factor I (IGFI) extracellular signaling genes were identified as being specifically upregulated (i.e. more significantly changed IGFI genes than expected by chance) by hedonic rough-and-tumble play but not depressogenic social defeat in the frontal and parietal cortices of rats. These results were corroborated by quantitative mRNA and protein assays. Direct lateral ventricle injections of IGFI or IGFI receptor (IGFIR) siRNA demonstrated that IGFI, through its action on the IGFIR, plays a functional role in the generation of positive affect as measured by hedonic USVs.

EXPERIMENTAL PROCEDURES

Animals

For microarray, quantitative real-time polymerase chain reaction (QRT-PCR) and protein studies, Long-Evans rats (LE; Harlan, USA) were used given that they exhibit robust conspecific rough and tumble play and social defeat behavior (Burgdorf et al., 2006; Kroes et al., 2007). For microinjection studies Fischer 344 × Brown Norway F1 (FBNF1) rats were used given that adult animals are required for cannulation surgery, and that FBNF1 rats exhibit robust heterospecific rough and tumble play in adulthood (unpublished observation). All rats were singly housed in Lucite cages with corn cob or sawdust bedding, maintained on a 12:12 light:dark cycle (lights on 8:00 am), and given ad libitum access to Purina lab chow and tap water throughout the study. All experiments were approved by either Bowling Green State University or Northwestern University Animal Care and Use Committees.

Focused microarray

The genes comprising the in-house rat CNS microarray were compiled from currently available NCBI/EMBL/TIGR rat sequence databases and commercially available CNS arrays (Clontech, Mountain View, CA, USA; Affymetrix, Santa Clara, CA, USA), and strategically chosen to provide representation of greater than 90% of the major gene ontological categories. Individual 45-mer oligonucleotides complementary to sequences of 1178 cloned rat CNS genes were spotted in triplicate onto slides. For a full description of the microarray, please see (Kroes et al., 2006, 2007).

Microarray analysis of gene expression. Microarray and data analysis were conducted as previously described in (Kroes et al., 2006, 2007). Microarray analyses were performed on tissues from individual animals in duplicate. Total mRNA was isolated from the frontal cortex and parietal cortex separately for individual rats (RNAseq, Qiagen, USA), and converted to cDNA (SuperScript® III, Invitrogen, USA). QRT-PCR was performed using Brilliant SYBR Green QRT-PCR Master Mix (Stratagene, La Jolla, CA, USA) on an Mx3000P Real-Time PCR System with ROX reference dye. Original input cDNA amounts were calculated by comparison to standard curves using purified-PCR product as a template for the mRNAs of interest.

The sequences of the QRT-PCR primers used in the study were as follows:

- IGFI (NM_178866), forward 5'-GACACACAAAGAAGGGAACG-3' and reverse 5'-CTGTAGCTTGTGGTCCCTG-3'.
- IGFIR (NM_052807), forward 5'-CCCAACAGGTCCCTACACG-3' and reverse 5'-AGTCCGCTCTCTAGATCTC-3'.
- IGFBP1 (NM_013144), forward 5'-GGTTCTCAGATGAGAGCACA-3' and reverse 5'-TGAGGTGAACCTACCTCCAGA-3'.
- IGFBP2 (NM_013122), forward 5'-GGTCTCTTGCAACACTCCT-3' and reverse 5'-GTCATGAGACATCCTTGCC-3'.
- IGFBP3 (NM_012588), forward 5'-ACAGACACCCAGAAGCTCTTC-3' and reverse 5'-GCAACATTGAGGAGCTCC-3'.
- IGFBP4 (NM_001004274), forward 5'-CAAGGTTGATGCAAGGAG-3' and reverse 5'-GGTGAACGTGGTGTG-3'.
- IGFBP5 (NM_012017), forward 5'-TACTCTGCCCACCTGAC-3' and reverse 5'-GCTTCATGCATATCC-3'.
- IGFBP6 (NM_013104), forward 5'-AGGCCCATGCTCTGTTCAA-3' and reverse 5'-GCTTGGGGTAAAGACCTCG-3'.

Protein determinations

For IGFI protein quantification, brain tissue was extracted in 0.1 N HCl, and total protein content was determined by the Lowry method. The iodinated IGFI peptide and antibody for IGFI were obtained from Bachem (UK), and radioimmunoassay was performed as previously described in (Burgdorf et al., 2006). IGFI samples were either assayed immediately after extraction or stored at 4 °C until assay. For IGFIR protein quantification, brain tissue was extracted in RIPA buffer (Millipore, USA) with 0.1% protease inhibiter cocktail (Sigma, USA), total protein content was determined by BCA (Pierce, USA), and IGFIR levels were quantified by ELISA (STAR IGF-1R, Millipore, USA) using the protocol recommended by the manufacturer. For IGFBP2 protein quantification, brain tissue was extracted in lysis buffer (10 mM Tris (PH 7.4), 1 mM Na3VO4, 1% SDS) with 0.1% protease inhibitor cocktail (Sigma, USA), total protein content was determined by BCA (Pierce, USA), and samples were analyzed by SDS-PAGE. Samples (50 μg) and a dilution series (1:1) of pooled samples (12.5–100 μg) were loaded onto 10% polyacrylamide gels (Hoefer, USA), transferred onto PVDF membranes (Millipore, USA), and probed with a goat polyclonal IGFBP2 antibody (sc-6002, Santa Cruz Biotechnology, USA) at 1:500 dilution overnight at 4 °C. Subsequent to 1 h incubation at 25 °C with 1:5000 dilution of HRP-conjugated secondary antibody (sc-2350, Santa Cruz Biotechnology, USA), IGFBP2 immunoreactivity was visualized by enhanced chemiluminescence (Immum-Star HRP, Biorad, USA) and development on film (BioMax, Kodak, USA) for appropriate times. The membranes were stained with Ponceau S (Sigma,
USA) and IGFBP2 levels were normalized to a prominent ~52 kDa band. Images were quantified by ImageJ (NIH).

Behavioral studies

Play and social defeat. For the rough-and-tumble play studies, 32-day-old LE rats of both genders were individually placed into the homecage of a conspecific rat for 30 min (experimental group n = 12 for mRNA studies, n = 8 for protein studies) or alone in a conspecific’s homecage (control group n = 6 for mRNA studies, n = 8 for protein studies). All animals in the play experiments remained isolate housed after weaning at PND 21. Chronic social isolation is critical to elicit rapid and robust rough-and-tumble play behavior (Panksepp et al., 1984; Ikemoto and Panksepp, 1992; Burgdorf et al., 2006). Pilot data pairing an adult female with an adolescent animal to control for social and somatosensory stimuli associated with play elicited high rates of play behavior and 50-kHz USVs (data not shown) and therefore this group could not be used. To control for the social and somatosensory stimuli associated with play, 32-day-old LE rats of both genders selectively bred for high rates of hedonic USVs (Burgdorf et al., 2009) received 20 min of experimenter delivered rough-and-tumble play behavior (heterospecific play; experimental group n = 10) or 20 min of experimenter delivered light touch (control group n = 8) using a 15 s stimulation followed by 15 s no stimulation protocol across the 20 min test session as described in (Burgdorf and Panksepp, 2001). Animals were sacrificed 6 h after testing, with their brains rapidly dissected (~90 s), frozen on dry ice, and stored at ~80 °C until assayed as described in (Burgdorf et al., 2006). The 6 h timepoint was chosen given that we have previously reported robust changes in brain mRNA and protein levels following play or social defeat (Panksepp et al., 2004, 2007; Burgdorf et al., 2006; Kroes et al., 2006, 2007). To control for high arousal levels associated with play behavior, an additional social defeat (high arousal with negative affect) group was included. For the social defeat study, 3-month-old adult male LE rats were placed into the homecage of a 6-month-old aggressive male LE rat for 30 min (experimental group n = 8) or a non-aggressive 3-month-old male LE rat (control group n = 8). To control for social interaction in the above social defeat study, data from Kroes et al. (2006) was reanalyzed comparing animals receiving 30 min of social defeat compared to 30 min alone in a conspecific’s homecage. Previous studies have shown that brain IGFI protein levels vary by age (García-Segura et al., 1991), and are increased by exercise (Trejo et al., 2001). To further control for age, arousal, somatosensory stimulation, and locomotor activity associated with play, basal Cortical IGFI protein levels were measured in adult male rats (3 month old) selectively bred for high, random, and low rates of play induced 50-kHz USVs (Burgdorf et al., 2006). These selectively bred lines do not differ in rates of spontaneous locomotor behavior (Burgdorf et al., 2006).

Animals in the play and social defeat studies were sacrificed 6 h after testing. Adult animals selectively bred for high, random, and low rates of hedonic 50-kHz USVs (Burgdorf et al., 2009) were sacrificed without prior behavioral testing. After sacrifice, the brains were rapidly removed (~90 s), dissected at 4 °C as described in (Burgdorf et al., 2006), and stored at ~80 °C until mRNA or protein extraction as described below. Each brain region was assayed separately for all biochemical tests.

Microinjection studies. Male 3-month-old FBNF1 animals were used in these studies. Adult animals were required for this study given that chronic indwelling cannulae and head caps inhibit play in juvenile animals (unpublished observation). FBNF1 animals were chosen given that this strain shows high stables rates of heterospecific play-induced hedonic USVs and reward as adults. Unilateral 22-gauge guide cannulae (Plastic Products, USA) were implanted into the lateral ventricle under isoflurane anesthesia and secured to the skull with jewelers’ screws and dental cement as described in (Burgdorf et al., 2007).

RESULTS

Ultrasonic vocalization recording and analysis

Methods used were identical to (Burgdorf et al., 2008). High frequency recordings of ultrasonic vocalizations were captured using a condenser microphone amplified by a bat detector (D980, Pettersson Elektronik, Sweden) and recorded with a Fostex FR2 field recorder (192 kHz sampling rate, 24 bit) onto compact flash cards (SanDisk, USA) as.wav files. Ultrasonic vocalizations were scored manually in a blind manner from sonograms generated by Avisoft SAS Lab Pro (Germany). Hedonic 50-kHz USVs, defined as having a peak frequency of greater than 40-kHz and a bandwidth greater than 18-kHz (i.e. frequency modulated), were scored (Burgdorf et al., 2008). Aversive 20-kHz USVs, defined as having a peak frequency of 20–30 kHz and a call duration greater than 500 ms, were also scored (Burgdorf et al., 2008). High inter- and intra-rater reliability for these measures (pearson’s r = 0.90) has been established for this method (Burgdorf et al., 2008).

Statistical analysis

Microarray data were analyzed by SAM with a false discovery rate cutoff of 10%, and gene families were analyzed querying the Gene Ontology database via GoMiner and χ² analysis. QRT-PCR, protein, and behavioral data were analyzed with ANOVA followed by Fisher PLSD post hoc tests, and false discovery rates based on 5000 random permutations for individual comparisons were also calculated for QRT-PCR and protein data as described in (Panksepp et al., 2007). All α = 0.5.
control group ($P>0.05$). Similarly, GoMiner analysis (Harris et al., 2004) revealed a significant enrichment in the IGF receptor signaling pathway (GO ID #48009) in the co-expressed genes at 6 h after play ($P<0.005$), but not following social defeat ($P>0.05$).

As summarized in Fig. 1, rough-and-tumble play elicited more hedonic USVs and lower rates of aversive 20-kHz USVs than social defeat as indexed by a significant condition×vocalization interaction ($F(1,15)=205.6$, $P<0.0001$) followed by Fisher PLSD post hoc tests comparing play vs. social defeat for both hedonic 50-kHz and aversive 20-kHz USVs ($P<0.0001$).

**QRT-PCR**

Play increased mRNA levels of IGFI, IGFIR, IGFBP1-3, and IGFBP5 in the frontal and posterior cortex as measured by QRT-PCR and indexed by a significant main effect for play ($F(1,142)=7.1$, $P<0.01$) followed by significant post hoc tests comparing play vs. control for IGFI, IGFIR, IGFBP1-3, and IGFBP5 (Table 3). The false discovery rate for each of the significant comparisons was less than 5%. Social defeat did not significantly alter mRNA levels of any of the IGFI signaling genes in the frontal and posterior cortex as measured by QRT-PCR ($F(1,98)=1.0$, $P>0.05$).

**Protein determinations**

Play increased IGFI protein levels compared to age matched controls ($F(1,14)=14.9$, $P<0.005$) and social defeat decreased IGFI protein levels compared to age matched controls ($F(1,14)=14.9$, $P<0.005$).

**Table 2. Changed genes in the frontal and parietal cortex 6 h after social defeat**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Frontal cortex</th>
<th>Parietal cortex</th>
<th>Gene ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calmodulin 3</td>
<td>1.10</td>
<td>0.90</td>
<td>Calmodulin 3</td>
</tr>
<tr>
<td>Prk</td>
<td>1.15</td>
<td>0.72</td>
<td>Prk</td>
</tr>
<tr>
<td>Atxcp2</td>
<td>1.18</td>
<td>0.38</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1. Changed genes in the frontal and parietal cortex 6 h after rough-and-tumble play**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Frontal cortex</th>
<th>Parietal cortex</th>
<th>Gene ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calmodulin 3</td>
<td>0.92</td>
<td>1.16</td>
<td>Calmodulin 3</td>
</tr>
<tr>
<td>Glial fibrillary acidic protein</td>
<td>1.06</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>Tubulin beta 5</td>
<td>1.12</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>Vimentin</td>
<td>0.91</td>
<td>1.16</td>
<td></td>
</tr>
<tr>
<td>Insulin-like growth factor receptor signaling pathway</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin-like growth factor 1</td>
<td>1.11</td>
<td>1.13</td>
<td></td>
</tr>
<tr>
<td>Insulin-like growth factor binding protein 3</td>
<td>1.06</td>
<td>1.13</td>
<td></td>
</tr>
<tr>
<td>Neuronal pentraxin II</td>
<td>1.04</td>
<td>1.08</td>
<td></td>
</tr>
<tr>
<td>Solute carrier family 1 member 3</td>
<td>1.09</td>
<td>1.09</td>
<td></td>
</tr>
<tr>
<td>Carboxy peroxidase E</td>
<td>1.08</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>Neurotrophic tyrosine kinase receptor type 3</td>
<td>1.11</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>Peptidylprolyl isomerase A</td>
<td>0.88</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>Prion protein</td>
<td>1.06</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>Syntaxin 8</td>
<td>1.05</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Guanine nucleotide binding protein, beta 1</td>
<td>0.91</td>
<td>1.09</td>
<td></td>
</tr>
<tr>
<td>Interleukin 18</td>
<td>1.07</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td>Proteolipid protein</td>
<td>0.91</td>
<td>1.12</td>
<td></td>
</tr>
</tbody>
</table>

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*The fold change (experimental/control) was calculated between mean values of social defeat (n=8) and control (n=6) rats.
Table 3. Play but not social defeat increases mRNA levels of the IGFI family of genes by QRT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control</th>
<th>Play</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGFI</td>
<td>5.21±0.23 E-07</td>
<td>6.28±0.31 E-07</td>
<td>0.0371</td>
</tr>
<tr>
<td>IGFIR</td>
<td>2.55±0.28 E-05</td>
<td>3.55±0.30 E-05</td>
<td>0.0486</td>
</tr>
<tr>
<td>IGFBP1</td>
<td>6.15±0.64 E-08</td>
<td>9.16±0.81 E-08</td>
<td>0.0270</td>
</tr>
<tr>
<td>IGFBP2</td>
<td>3.01±0.28 E-05</td>
<td>3.97±0.26 E-05</td>
<td>0.0335</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>4.33±0.33 E-06</td>
<td>5.78±0.38 E-06</td>
<td>0.0270</td>
</tr>
<tr>
<td>IGFBP4</td>
<td>2.27±0.22 E-05</td>
<td>2.59±0.24 E-05</td>
<td>0.0444</td>
</tr>
<tr>
<td>IGFBP5</td>
<td>5.21±0.23 E-06</td>
<td>6.28±0.31 E-06</td>
<td>0.0371</td>
</tr>
<tr>
<td>IGFBP6</td>
<td>1.60±0.14 E-05</td>
<td>1.97±0.21 E-05</td>
<td>0.0708</td>
</tr>
</tbody>
</table>

Gene Control Social defeat $P$ value

IGFI 3.87±0.20 E-07 4.14±0.29 E-07 0.4609
IGFIR 2.42±0.16 E-05 2.68±0.20 E-05 0.3345
IGFBP1 8.49±0.41 E-08 1.04±0.09 E-07 0.0649
IGFBP2 4.44±0.5 E-05 4.76±0.66 E-05 0.6991
IGFBP3 1.90±0.16 E-06 2.18±0.23 E-06 0.3252
IGFBP4 2.80±0.20 E-05 3.49±0.50 E-05 0.2224
IGFBP5 4.73±0.40 E-06 5.46±0.55 E-06 0.2969
IGFBP6 1.97±0.12 E-05 2.27±0.18 E-05 0.1801

* QRT-PCR data is expressed as mean±SEM pg transcript/pg CDNA.

** Fisher PLSD post hoc test comparing play (n=6) versus control (n=12) or social defeat (n=8) versus control (n=8). All significantly changed genes (i.e. $P<0.05$) have a false discovery rate of less than 5%.

matched controls ($F(1,14)=5.1, P<0.05$) in both the frontal and parietal cortex, as measured by radioimmunoassay (Fig. 2).

To control for social and somatosensory stimulation associated with play, IGFI levels were also measured following experimenter delivered heterospecific play vs. light touch (Burgdorf and Panksepp, 2001). Rates of hedonic USVs were significantly elevated in the heterospecific play group (Mean±SEM vocalizations/20 min) 1791.4±50.3 as compared to the light touch group 6.4±3.1 ($F(1,14)=1252.8, P<0.0001$). Higher IGFI protein levels in the frontal and posterior cortex 6 h after testing were exhibited in the heterospecific play group ($F(1,15)=8.2 P<0.05$) as compared to the light touch group (Fig. 2).

To further control for age, social interaction, and somatosensory stimulation associated with play, basal IGFI protein levels were measured in the frontal cortex of animals selectively bred for differential rate of play induced 50-kHz USVs (Burgdorf et al., 2009) that received no behavioral testing before sacrificed. Animals bred for high rates of 50-kHz USVs had higher basal IGFI protein levels compared to either random or low line animals ($F(2,20)=7.0, P<0.01$) followed by significant post hoc tests comparing high line vs. random line or high line vs. low line ($P<0.01$; Fig. 3).

As depicted in Fig. 4, 6 h after play testing, IGFIR protein levels in the frontal and posterior cortex were significantly elevated as compared to controls as measured by ELISA ($F(1,14)=16.3, P<0.005$). IGFBP2 is the most abundant IGFI binding protein in the brain (Ocrant et al., 1990) and was therefore chosen for protein validation studies. Play also increased IGFBP2 protein levels at 6 h after play testing as compared to controls, both in the frontal and posterior cortex, as measured by Western analysis ($F(1,14)=26.2, P<0.010$).

**IGFI microinjections**

As reported in Fig. 5, microinjections of IGFI into the lateral ventricle increased rates of hedonic USVs, and this effect
was blocked by a silent dose of the IGFIR antagonist JB1, as indexed by a main effect for drug ($F(3,36)=4.1$, $P<0.05$) followed by significant Fisher PLSD post hoc comparisons ($P<0.05$) between the IGFI group and all other groups. In a similar manner, IGFI also increased the rewarding value of play as measured by approach speed ($F(3,36)=8.8$, $P<0.0005$; post hoc tests comparing IGFI to all other groups $P<0.05$).

siRNA knockdown of IGFIR

Lateral ventricle infusions of an IGFIR-specific siRNA decreased rates of play-induced hedonic USVs ($F(1,27)=4.3$, $P<0.05$), and decreased the rewarding value of play as measure by approach speed ($F(1,27)=7.7$, $P<0.01$) compared to a scrambled siRNA control group (Fig. 6). IGFIR siRNA produced a (mean±SEM) 40.7±8.9% reduction in IGFIR protein levels in the frontal cortex as measured by ELISA ($F(1,27)=4.4$, $P<0.05$) with the control siRNA animals exhibiting (mean±SEM) 0.36±0.06 ng IGFIR/mg total protein.

DISCUSSION

These results show that IGFI plays a functional role in the generation of positive affective states as indexed by hedonic 50-kHz USVs. Hedonic rough-and-tumble play increased cortical mRNA and protein levels of IGFI, IGFIR, and IGFBP2. Positive affect was shown to specifically elevate cortical IGFI protein levels controlling for social interaction, somatosensory stimulation, age, and arousal. Cortical IGFI protein levels were also shown to be elevated in rats bred for high rates of hedonic USVs in a locomotor activity independent manner. Conversely, negative affective stimuli (i.e., social defeat) reduced cortical IGFI protein levels. IGFI was shown to promote the generation of hedonic USVs via its action on the IGFIR using direct lateral

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**Fig. 4.** Rough-and-tumble play increases protein levels of the IGFIR and IGFBP2. Mean±SEM fold change (play/control) in IGFIR protein levels as measured by ELISA and IGFBP2 as measured by Western analysis in the frontal and parietal cortex of rats 6 h after either 30 min of conspecific rough-and-tumble play or 30 min alone in a novel cage (control). ** $P<.01$, *** $P<.001$ Fisher PLSD post hoc test. The false discovery rate for both IGFIR and IGFBP2 were less than 1%. n=8 per group.

**Fig. 5.** Direct injection of IGFI increases rough-and-tumble play-induced hedonic USVs, the rewarding value of play, and is anxiolytic. Mean±SEM play-induced hedonic USVs (a), the rewarding value of play as measured by approach latency to self-administer play (b) and anxiolytic effect of play as measured by open field center crosses (c) following lateral ventricle infusions of IGFI ($2\mu$g), IGFI antagonist JB1 ($30\mu$g) or dH2O vehicle in $2\mu$l total volume at $1\mu$l/min 20 min before the start of testing. The silent dose of JB1 blocked IGFI effects for all three behavioral measures. * $P<.05$ Fisher LSD post hoc test, IGFI vs. all other groups. n=10 per group.
ventricle injections of a IGFIR specific siRNA (35 pmol; latency for the animal to self administer play 1–2 days after lateral ventricle administration of IGFI alone and in concert with a silent dose of the IGFIR antagonist JB1. Lastly, lateral ventricle infusions of an IGFIR-specific siRNA diminished hedonic USVs compared to scrambled siRNA-injected controls.

In humans, IGFI has been shown to be elevated by positive affective states and induce resilience to depression and anxiety. IGFI, a 7 kDa peptide produced primarily in the liver, binds to an IGFI-specific tyrosine kinase receptor (IGFIR; Russo et al., 2005) and has been shown to cross the blood-brain barrier (Reinhardt and Bondy, 1994). In growth hormone-deficient patients, growth hormone-induced elevation of IGFI has been shown to increase positive affective states and decrease rates of depression (Stouthart et al., 2003; Lasaite et al., 2004). In these same studies, increases in positive affect and decreases in depression scores are positively correlated with IGFI plasma levels (Stouthart et al., 2003; Lasaite et al., 2004). In addition, i.v. IGFI injections reduce levels of depression and anxiety in humans (Thompson et al., 1998; Graham et al., 2007).

In rats, IGFI has been shown to be elevated by hedonic exercise and induce resilience to depression and anxiety. The antidepressant effect of voluntary exercise in rats appears in part to be mediated by exercise-induced increases in brain IGFI levels (Trejo et al., 2008). These effects have been found to be IGFIR-specific given that the administration of an anti-IGFI antibody blocks the antidepressant effects (Duman et al., 2009). IGFI administration also has antidepressant effects in the forced swim test and tail suspension test, and anxiolytic effects in the elevated plus-maze test in both rats and mice (Hoshaw et al., 2005; Malberg et al., 2007, Fig. 5).

Positive affect also increases cortical levels of the key IGFI extracellular signaling genes. In this study, play increased both mRNA and protein levels of IGFI, IGFIR, and IGFBP2 (the most abundant binding protein in the brain; Ocrant et al., 1990). Although IGFI mRNA was increased in the brain following rough-and-tumble play, it is uncertain if the increase in IGFI protein levels was due primarily to increased brain permeability or de novo synthesis. IGFI levels are approximately 10-fold higher in plasma than in the cerebrospinal fluid (Poljakovic et al., 2006). The increase in IGFBP2 seen following play may contribute to increased IGFI brain bioavailability (Reinhardt and Bondy, 1994). While IGFI binding proteins do compete with the IGFIR for IGFI binding, they have also been shown to increase the half life of IGFI, and in some cases chaperone IGFI to target tissues (Russo et al., 2005). Further, it has been shown that IGFBP2 facilitates IGFI-induced neurogenesis (Brooker et al., 2000).

Although these results indicate that IGFI-induced positive affect is IGFIR-dependent, multiple IGFI-related molecular mechanisms appear to participate in IGFI induction of positive affective states. IGFI, through its action on the IGFIR, has been implicated both in rapid neuronal changes such as fast neuronal signaling through voltage-gated calcium channels (Blair and Marshall, 1997), as well as slower processes such as neurogenesis and neuronal growth (Russo et al., 2005). The antidepressant effect of IGFI has been shown both minutes and days after injection, suggesting that both short- and long-term mechanisms may play a role (Hoshaw et al., 2005; Malberg et al., 2007). In the present study, play was shown to acutely increase IGFI levels, and IGFI was shown to acutely increase positive affective states. In rats, there is a positive correlation between levels of heterospecific play induced 50-kHz USVs and hippocampal neurogenesis (Wohr et al., 2009). IGFI may also play a role in positive affect induced facilitation of neurogenesis. Further studies examining IGFIR-dependent signaling are required to determine the precise molecular mechanism(s) of IGFI-induced positive affect.

These results support the idea that IGFI is a sufficient but perhaps not a necessary part of the molecular mechanism(s) that lead to the induction of positive affective states. Other growth factors have also been shown to be elevated by positive affective states and/or induce resilience to depression and anxiety (Gordon et al., 2003; Stouthart et al., 2003; Hunsberger et al., 2007; Turner et al., 2008). Additionally, both the mesolimbic dopamine system and opioids play a functional role in the generation of positive affective states (Burgdorf and Panksepp, 2006; Le Merrer et al., 2009).

CONCLUSION

In sum, while positive affective states have been shown to induce resilience to depression in humans, currently available antidepressants do not adequately treat the anhedonic symptoms of depression (Nierenberg et al., 1999, Nutt et al., 2007). The present results demonstrate that IGFI
plays a functional role in the generation of positive affective states as measured by hedonic 50-kHz USVs. The development of drugs to promote IGF-I signaling pathway in addition to other growth factors already identified (Duman and Monteggia, 2006; Turner et al., 2006; Aki et al., 2008), may yield new treatments to ameliorate the anhedonic symptoms of depression.

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