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Effects of Interferon-alpha on Rhesus Monkeys: A Non-Human Primate Model of Cytokine-Induced Depression

Jennifer C. Felger, **Oyetunde Alagbe**, **Fang Hu**, **Deborah Mook**, **Amanda A. Freeman**, **Mar M. Sanchez**, **Ned H. Kalin**, **Emiliangelo Ratti**, **Charles B. Nemeroff**, and **Andrew H. Miller**
From the Department of Psychiatry and Behavioral Sciences (JCF, OA, FH, MMS, CBN, AHM), the Winship Cancer Institute (JCF, OA, FH, AHM), the Center for Behavioral Neuroscience (JCF, MMS), the Yerkes National Primate Research Center (MMS), Division of Animal Resources (DM), and the Department of Neurology (AAF), Emory University School of Medicine, Atlanta, Georgia; the Department of Psychiatry (NHK), University of Wisconsin-Madison, Madison, Wisconsin; and the Psychiatry Centre of Excellence for Drug Discovery (ER), Pharmaceuticals R&D, GlaxoSmithKline, Verona, Italy

Abstract

Background—Interferon (IFN)-alpha is an innate immune cytokine that causes high rates of depression in humans and therefore has been used to study the impact of cytokines on the brain and behavior. To establish a non-human primate model of cytokine-induced depression, we examined the effects of IFN-alpha on rhesus monkeys.

Methods—Eight rhesus monkeys were administered recombinant human (rHu)-IFN-alpha (20 MIU/m^2) or saline for 4 weeks in counterbalanced fashion, and videotaped behavior, as well as plasma and cerebrospinal fluid (CSF), were obtained at regular intervals to assess behavioral, neuroendocrine, immune and neurotransmitter parameters. Additionally, expression and activity of IFN-alpha/beta receptors in monkey peripheral blood mononuclear cells (PBMCs) were assessed.

Results—Compared to saline treatment, IFN-alpha administration was associated with persistent increases in anxiety-like behaviors and decreases in environmental exploration. In addition, IFN-alpha induced significant increases in plasma concentrations of ACTH, cortisol, and interleukin-6 that tended to diminish after chronic administration, especially in dominant animals. Interestingly, in 3 animals, depressive-like, huddling behavior was observed. Monkeys that displayed huddling behavior exhibited significantly higher plasma concentrations of ACTH and lower CSF concentrations of the dopamine metabolite, homovanillic acid. Rhesus monkey PBMCs were found

Corresponding author: Andrew H. Miller, MD, Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Winship Cancer Institute, 1365-C Clifton Rd, 5th floor, Atlanta, GA 30322 (e-mail: amill02@emory.edu).

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to express mRNA and protein for the IFN-alpha/beta receptor. Moreover, treatment of PBMCs with rHu-IFN-alpha led to induction of STAT1, one of the primary IFN-alpha-induced signaling molecules.

Conclusions—IFN-alpha evoked behavioral, neuroendocrine and immune responses in rhesus monkeys that are similar to humans. Moreover, alterations in hypothalamic-pituitary-adrenal axis responses and dopamine metabolism may contribute to IFN-alpha-induced depressive-like huddling behavior.

Keywords

Interferon-alpha; HPA-axis; proinflammatory cytokines; behavior; non-human primate; animal model; dopamine

INTRODUCTION

Interferon (IFN)-alpha is an innate immune cytokine released in response to viral infection that is used to treat infectious diseases and cancer (1,2). Although an effective therapy, IFN-alpha is associated with significant depressive symptoms in up to 50% of patients, depending on the dose (3-7). Therefore, IFN-alpha has been used to investigate neurobiological pathways whereby cytokines influence the brain and behavior.

Multiple pathways appear to contribute to depressive symptoms during IFN-alpha treatment. In terms of effects on neuroendocrine and neurotransmitter function, IFN-alpha has been found to stimulate corticotropin-releasing factor (CRF) and the hypothalamic-pituitary-adrenal (HPA)-axis (8-11), and alter monoamine metabolism (12-16). In rodents, IFN-alpha has been found to increase CRF protein expression in the hypothalamus and amygdala (9,10) and to induce CRF release from hypothalamic explants (9). In humans, IFN-alpha activation of CRF pathways is manifested by increases in plasma ACTH and cortisol concentrations (8,9,11). Interestingly, the magnitude of IFN-alpha-induced stimulation of ACTH and cortisol following the first injection of IFN-alpha in patients with malignant melanoma was found to be associated with the subsequent development of depression during IFN-alpha treatment, suggesting that sensitization of CRF pathways may represent a vulnerability to IFN-alpha-induced depression (8,9).

Laboratory animal studies also have shown that IFN-alpha decreases brain concentrations of serotonin (5-HT) and dopamine (DA) (12-15). Effects of IFN-alpha on 5-HT pathways may be related to activation of the enzyme indoleamine-2,3-dioxygenase (16) which catabolizes tryptophan (TRP), the primary amino acid precursor of serotonin, to kynurenine and quinolinic acid. Moreover, IFN-alpha can activate p38 mitogen activated protein kinase which has been shown to increase expression and activity of the serotonin transporter (17,18). Finally, IFN-alpha can directly bind to opioid receptors, which can influence DA neurotransmission (19-21).

To further explore the pathophysiology and treatment of cytokine-induced behavioral alterations, we endeavored to develop an animal model of IFN-alpha-induced behavioral change using rhesus monkeys. Rhesus monkeys display a complex behavioral repertoire and have similar immune and neuroendocrine systems to humans. In addition, acute administration of IFN-alpha has been shown to alter sleep architecture in rhesus monkeys (22); consistent with reports of sleep disruption in IFN-alpha-treated patients (23).

The present study examined behavioral, neuroendocrine, immune, and neurotransmitter responses to 4 weeks of recombinant human (rHu)-IFN-alpha (20 MIU/m^2) compared to saline in rhesus monkeys. In addition, the expression of interferon-alpha/beta receptor 1 (IFNAR1)

and the ability of rHu-IFN-alpha to activate relevant IFN-alpha signaling pathways (phospho-STAT1 activation) was assessed in rhesus monkey peripheral blood mononuclear cells (PBMCs).

METHODS

Animals

Four male (5-10kg) and 4 female (5-7kg), rhesus monkeys (*Macaca mulatta*), aged 4-6yrs, were housed in same-sex, dominate-subordinate pairs either together (n=6) or individually in adjacent cages (n=2). Social status (dominant versus subordinate) was stable throughout the study, and was based on experimenter interactions, competition for hand-fed treats, mounting, and occasional aggression. Animals were fed Purina monkey chow twice daily and were maintained on a 7am-7pm light-dark cycle. Although food intake was not monitored, body weight of the monkeys did not change during saline or IFN-alpha administration [delta weight, week4-week1: 0.044kg (SD 0.038) for saline versus 0.013kg (SD 0.051] for IFN-alpha, p=NS]. All study procedures were approved by the Emory Institutional Animal Care and Use Committee or the Emory Institutional Review Board. Human subjects who donated blood provided written informed consent.

Design

A counterbalanced design was employed. For each dominant-subordinate pair, one received IFN-alpha and the other received saline during one of two treatment periods. The treatment conditions were reversed after 3 months such that each animal received both IFN-alpha and saline. Prior to treatment initiation, animals were habituated to study procedures in order to minimize stress reactivity. IFN-alpha [rHu-IFN-alpha-2b (Schering-Plough, Kenilworth, NJ)] 20 MIU/m^2 or saline was administered subcutaneously in equivalent volumes (0.5-1ml) between 7-9am, 5 days per week for 4 weeks, similar to the treatment schedule of patients receiving IFN-alpha monotherapy for malignant melanoma (5). Plasma and CSF were collected 3hrs post-IFN-alpha or saline administration on the first day of weeks 1 (initial injection), 2, and 4. Behavior was sampled on the first or second days of each week, 2hrs post IFN-alpha or saline administration.

Behavior

Videotaped behavior was scored in 30 minute observational periods by trained observers using a microprocessor-based syntactic behavioral scoring system (Observer, Noldus, Leesburg, VA) and operationally defined categories derived from a basic rhesus monkey ethogram (24). The ethogram included measures of locomotor, social, environmental exploratory, and anxiety-like behaviors. Because not all animals were housed in social pairs, data on social behaviors was not included in the analysis. Behaviors were scored by two raters blinded to treatment condition. Inter-rater reliability was maintained at ≥85%. Scored behaviors were grouped into 3 categories, including anxiety-like behaviors, exploratory behaviors, and locomotor activity. Anxiety-like behaviors included self-scratching, body-shakes, and yawning; behaviors sensitive to stress and benzodiazepine administration in monkeys (25-28). Environmental exploration consisted of tactile or oral manipulation of surroundings such as foraging for food, manipulation of cage or toys. Locomotor activity consisted of walking, running, and jumping. Huddling, a measure of depressive-like behavior in monkeys (29-32), was also assessed.

Sample Collection

Blood and CSF were obtained under Telazol (3-5mg/kg) anesthesia within 5-10min (blood) or 10-20min (CSF) of initial experimenter contact. Blood was obtained via femoral venipuncture, collected in chilled EDTA tubes and centrifuged at 1000×g for 15min at 4°C for removal of

plasma. Plasma was replaced by saline, and PBMCs were isolated by density gradient centrifugation. CSF was collected passively into chilled polypropylene tubes by inserting a 22-23gauge needle into the *cisterna magna* following published protocols (33,34), and frozen on dry ice. Plasma and CSF were stored at -80°C until assayed.

Assays

Commercially available immunoradiometric assay (IRMA) and radioimmunoassay (RIA) kits were used for assessment of plasma ACTH (ALPCO Diagnostics, Salem, NH, and Nichols Institute Diagnostics, San Juan Capistrano, CA when available) and cortisol (DiaSorin Stillwater, MN), respectively. Assay sensitivities are 1.0pg/ml (ALPCO) or 0.5pg/ml (Nichols) for ACTH and 0.12μg/dl for cortisol. Intra- and inter-assay CVs respectively are 2.8% and 5.7% (ALPCO) or 4.5% and 6.3% (Nichols) for ACTH and 8.5% and 12.7% for cortisol. Plasma and CSF IL-6 and IL-1beta were measured using sandwich enzyme-linked immunosorbent assays (ELISAs) according to manufacturer's protocol (R&D Systems, Minneapolis, MN). The sensitivities of these assays are <0.7pg/ml and <0.1pg/ml, and the intra- and inter-assay CVs respectively are 2.6% and 4.5% for IL-6 and 7.8% and 12.6% for Il-1beta. Plasma and CSF IFN-alpha was measured using a sandwich ELISAs (Bender Med Systems, Burlingame, CA). Assay sensitivity is 56pg/ml for plasma and 12.5pg/ml for CSF, and the intra- and inter-assay CVs are <5% and <10% respectively. Of note, the ACTH IRMAs and cytokine ELISAs employed were developed for use in humans, however these kits have been extensively used in previous studies on rhesus monkeys (35-39). Moreover, there is a high amino acid sequence homology in human and rhesus monkey IL-1beta, Il-6, and IFN-alpha (cytokines measured in this study) ranging from 95.9 to 98.1% (40).

Homovanillic acid (HVA), 3,4-dihydroxy-phenylacetic acid (DOPAC), and 5-hydroxyindole acetic acid (5-HIAA) were measured in duplicate 20μl CSF aliquots using high performance liquid chromatography as described previously (41). Electrochemical detection was performed using a dual electrode system (ESA Inc., Chelmsford, MA) set at oxidative potentials of 300 and 450mV (HVA, DOPAC) and 150 and 300mV (5-HIAA). Peak areas were computed using Turbochrom software (PE-Nelson, San Jose, CA). Sensitivities for all analytes are ~1pg on column.

CRF was measured in 100¼l of CSF using a CRF RIA as previously described (42). The anti-CRF antisera (IgG Corporation, Nashville, TN) was developed in rabbits against the N-terminal portion of the intact CRF peptide coupled to human γ-globulins with bisdiazotized benzidine. Samples were assayed in triplicate, the limit of detection is 3.9pg/ml, and the intra-assay and inter-assay CVs are 6.1% and 10.2%, respectively.

RT-PCR for IFNAR1

RNA was isolated from IFN-alpha-naïve monkey and human PBMCs and reverse-transcribed with SuperScriptt First-strand Synthesis System for RT-PCR (Invitrogen, Carlsband, CA, USA) using random primers, according to manufacturer's protocols. Primers corresponding to regions in the human IFNAR1 open reading frame were used to amplify IFNAR1 cDNAs using Platinum PCR SuperMix (Invitrogen Corporation). Primer sequences were as follows: 5'-ATGATGGTCGTCCTCCTGGGC-3'(forward) and 5'-TCATACAAAGTCCTGCTGTAGTTC-3'(reverse).

Western Blot for pSTAT1 and IFNAR1

Protein (~50ug) extracted from IFN-alpha-naïve monkey and human PBMCs was separated by SDS-PAGE (12%) gel, transferred to nitrocellulose membranes, and incubated with rabbit anti-pSTAT1(Tyr701) (Cell Signaling Technology, Beverly, MA) or goat-anti-human-IFNAR1 (1:1000, R&D Systems) antibodies, followed by secondary (detection) antibodies.

Stripped blots were reprobed with anti-actin antibodies (Sigma, St. Louis, MO) for loading control.

Flow Cytometry for pSTAT1

Whole-blood (200μl) collected from IFN-alpha-naïve monkeys and humans, was incubated with 5000IU/ml rHu-IFN-alpha-A (R&D Systems) for 0,15,30, or 60min. After lysing of red cells, white blood cells were fixed, permeabilized, and incubated with 20μl PE-mouse-anti-STAT1(pY701)-monoclonal-antibody (BD Pharmingen, San Diego, CA) for flow cytometry (BD Biosciences, Cellquest and FACScalibur). Data were analyzed using Flojo software (Tree Star, Inc., Ashland, OR).

Cell Culture for pSTAT1 Induction

PBMCs (2×10^6) collected from IFN-alpha-naïve monkeys and humans were incubated with 1000 IU/ml rHu-IFN-alpha-A (R&D Systems) for 0,15,30, or 60min in duplicate.

Statistical Analyses

Repeated measures (RM) analyses of variance (ANOVA) was used to assess effects of treatment (IFN-alpha and saline), time (weeks 1,2,3, and 4) and their interactions for relevant variables. Sphericity and normality of were computed using Mauchly and Kolmogorov-Smirnov tests, respectively. In cases where data were not normally distributed, standard transformation procedures were used to achieve normality (log10 for huddling and IL-1beta; natural log for anxiety, locomotor activity, and ACTH; square root for exploratory behavior, IL-6, IFN-alpha). Post hoc analyses were conducted using the Student-Newman-Keuls test of significance. To summarize mean responses for measures that were not time dependent and to address differences in mean behavioral and biological responses across the treatment period between dominant and subordinate animals, summary tables were employed. Mean responses across treatment for all animals in saline versus IFN-alpha treatment groups were compared using paired t-tests, and two-way ANOVAs were used to examine the effects of treatment (IFN-alpha vs. saline), social status (dominant vs. subordinate) as well as their interaction. For comparing means of relevant variables in monkeys that did or did not display huddling behavior, non-parametric t-tests (rank sum) were employed due to the small number of animals per group. For all CSF assays, sample replicates with CVs>10 % or values that were determined to be outliers using the Grubbs' test for outliers were excluded from analysis: 9 of 49 for CRF due to high CVs and 2 of 32 for IL-1beta. To examine the relationship between HVA concentrations and huddling behavior as well as the relationship between locomotor activity in dominant and subordinate pairs, Pearson's correlation coefficients were employed. Because of the counterbalanced study design and small sample size, a systematic analysis of order effects for IFN-alpha/saline administration was not conducted. All tests of significance were 2-tailed with an alpha level of 0.05.

RESULTS

Behavioral Changes

Repeated Measures ANOVAs indicated that compared to saline, monkeys treated with IFN-alpha exhibited increased anxiety-like behaviors ($F[1,7]=7.81, p<0.05$) and decreased environmental exploration ($F[1,7]=10.21, p<0.05$) with no significant effects of time or a treatment by time interaction (Figure 1a,b). Post-hoc analyses revealed significantly increased anxiety-like behaviors at weeks 1 and 4 ($p<0.05$), while exploratory behaviors were not significantly different between IFN-alpha and saline at any specific time point. When mean behavioral responses to IFN-alpha and saline were compared across the treatment period (Table 1), paired t-tests revealed both an increase in anxiety-like behaviors ($t=3.08, df=7, p<0.05$) and

a decrease in environmental exploration (t=2.57,df=7,p<0.05). RM ANOVAs revealed that locomotor behavior was not affected by treatment or time (Figure 1c); however, when mean locomotor activity across treatment was analyzed as a function of social status, a significant interaction between treatment and social status was uncovered (F[1,12]=6.29,p<0.05). Dominant monkeys demonstrated significantly reduced locomotor activity and subordinate monkeys exhibited significantly increased locomotor activity during IFN-alpha administration (p<0.05). Because animals were tested in pairs (one on saline and the other on IFN-alpha), the relationship between locomotor activity in dominant and subordinate pairs during individual treatment trials was examined. No significant correlations were uncovered. In addition, no differences were found between dominant and subordinate animals in anxiety-like or exploratory behaviors. Interestingly, 3 monkeys (two dominant and one subordinate) displayed depressive-like huddling behavior during IFN-alpha administration (F[1,2]=19.30,p<0.05) that was not affected by time (Figure 2). Post-hoc tests indicated that huddling behavior was significantly increased in relevant animals across all 4 weeks of IFN-alpha administration (p<0.05).

Neuroendocrine and Immune Changes during IFN-alpha Administration

Repeated Measures ANOVAs indicated that compared to saline, IFN-alpha was associated with increased plasma ACTH (F[1,7]= 24.91,p<0.05), cortisol (F[1,7]=7.96,p<0.05), and IL-6 (F[1,7]=9.35, p<0.05). There was a significant effect of time (F[2,7]=5.92,p<0.05) and an interaction for cortisol (F[2,14]=8.26,p<0.05), reflected by a decrease over the course of IFN-alpha administration. An effect of time for IL-6 was also observed (F[2,24]=3.54,p<0.05). As noted in Figure 3, post-hoc analyses indicated that IFN-alpha administration was associated with significant increases in plasma ACTH and IL-6 in weeks 1 and 2 (p<0.05), but not week 3. Cortisol was increased at week 1 only (p<0.05). As shown in Figure 4, the pattern of ACTH, cortisol, and IL-6 responses to IFN-alpha over time was dependent on social status. In dominant animals, there were no effects of treatment or time alone, however treatment by time interactions were observed for both ACTH (F[2,6]=21.01,p<0.05) and cortisol (F[2,6] =8.17,p<0.05). Post hoc analyses confirmed that dominant monkeys had increases in plasma ACTH and cortisol concentrations compared to saline in week 1 of IFN-alpha treatment (p<0.05). In subordinate animals however, there was a main effect of treatment for plasma ACTH (F[1,3]=20.00,p<0.05), cortisol (F[1,3]=11.84,p<0.05), and IL-6 (F[1,3] =28.16,p<0.05) with no effect of time or an interaction, indicating no change in response over time. Post hoc analyses revealed that compared to saline, subordinate monkeys had persistent increases in plasma ACTH (p<0.05), cortisol (p<0.05), and IL-6 (p<0.05) across IFN-alpha treatment with statistically significantly increases in ACTH and cortisol at weeks 1 and 2 (p<0.05), and IL-6 at weeks 1-3 (p<0.05).

Paired t-tests of mean hormone and cytokine responses across IFN-alpha versus saline treatment (Table 2) revealed increased plasma ACTH (t=4.24,df=7,p<0.05), cortisol (t=2.82,df=7,p<0.05), IL-6 (t=3.68,df=7,p<0.05) and IFN-alpha (t=3.49,df=7,p<0.05). Two way ANOVAs were used to explore differences in mean responses in these hormone and cytokine variables as a function of social status. No significant interactions between treatment conditions and social status were found.

CSF Monoamines, CRF and Cytokines during IFN-alpha Administration

No significant effect of IFN-alpha on CSF concentrations of HVA, DOPAC, 5-HIAA or CRF was observed (Table 3). Mean CSF IFN-alpha concentrations were significantly increased during IFN-alpha administration compared to saline (t=3.22,df=3,p<0.05) (Table 3). IFN-alpha administration also was associated with increased CSF IL-1beta in 4 monkeys where data were available (t=5.67,df=3,p<0.05) (Table 3). No significant correlations were found among CSF concentrations of cytokines, monoamine metabolites and CRF.

Huddling Behavior and Plasma ACTH and CSF HVA Concentrations

Comparison of mean plasma hormone and cytokine concentrations across IFN-alpha treatment revealed increased ACTH responses in monkeys that displayed depressive-like huddling behavior compared to those that did not ($T=21.00, df=2,4, p<0.05$), but no difference in plasma cortisol or IL-6 (Figure 5) Monkeys that demonstrated huddling behavior also exhibited a significant decrease in CSF HVA concentrations ($T=21.00, df=2,4, p<0.05$) during IFN-alpha administration compared to those that did not (Figure 6a). In addition, there was a significant negative correlation between the mean time spent huddling and mean CSF HVA concentrations during IFN-alpha treatment in huddling animals ($r=-0.998, p<0.05$) (Figure 6b).

Expression and Activity of IFNAR1 in Rhesus Monkey PBMCs

Expression of IFNAR1 was detected in IFN-alpha naïve rhesus monkey and human PBMCs by RT-PCR and Western blot (Figure 5a). Furthermore, IFN-alpha activation of p-STAT1 in IFN-alpha naïve rhesus monkey and human PBMCs *in vitro* at 15,30, and 60min was confirmed using flow cytometry (Figure 5b) and Western blot (Figure 5c).

DISCUSSION

Compared to saline, administration of IFN-alpha to rhesus monkeys was associated with immune, neuroendocrine, and behavioral responses similar to that observed in humans. Behavioral changes included depressive-like huddling behavior (3 of 8 monkeys), increased anxiety-like behavior, decreased environmental exploration, and alterations in locomotor activity that depended on social status. IFN-alpha administration was also associated with increased plasma ACTH, cortisol, and IL-6, which diminished throughout 4 weeks of treatment, particularly in dominant animals. Interestingly, monkeys that exhibited huddling behavior had significantly higher plasma ACTH and lower CSF HVA concentrations. Finally expression and activation of IFNAR1 in rhesus monkey PBMCs was confirmed.

Previous studies have demonstrated that innate immune cytokines administered acutely to laboratory animals can induce behavioral changes consistent with those seen in IFN-alpha-treated monkeys including depressive- and anxiety-like behavior (43-48). However, the majority of animal studies examining cytokine effects on behavior have used acute or sub-chronic dosing strategies, and a dearth of studies have examined behavioral changes following chronic cytokine exposure, as would occur during chronic inflammatory illnesses. Interestingly, changes in behavior observed in this study demonstrate that chronic administration of the innate immune cytokine, IFN-alpha, leads to behavioral changes similar to those seen following acute administration, with limited adaptation over time. These data are consistent with persisting behavioral effects of IFN-alpha over long periods of administration in humans and suggest that chronic exposure to endogenous cytokines that may occur in the context of a variety of medical illnesses has the capacity to induce enduring states of behavioral pathology.

Whether IFN-alpha-induced behavioral changes are due to direct effects of IFN-alpha in the CNS has yet to be resolved. Previous data from rodents and monkeys have suggested that central penetration of peripherally administered IFN-alpha is low (49,50). Nevertheless, concentrations of IFN-alpha were increased in both plasma and CSF in this study, providing evidence that IFN-alpha may access the CNS and act centrally, although it should be noted that the ELISA used to measure IFN-alpha in this study recognizes multiple IFN-alpha subtypes, and therefore the source of the IFN-alpha (endogenous versus exogenous) cannot be definitively established. IFN-alpha may also exert indirect effects via induction of other peripheral and/or central cytokines. As demonstrated in this and other studies, IFN-alpha is a potent inducer of IL-6 (8,11,51,52), while also stimulating TNF-alpha and IL-1 (52,53). Indeed,

IL-1beta was detected in peripheral blood of IFN-alpha-treated animals, and a significant increase in IL-1beta was found in the CSF of a subset of animals. Taken together, these data suggest that IFN-alpha may penetrate the brain and activate central inflammatory responses including the production of IL-1.

Of relevance to the relationship between the behavioral effects of IFN-alpha in rhesus monkeys and depression in humans was the induction of huddling behavior. Huddling is a fetal-like, self-enclosed position with head at or below shoulders (29,31) thought to reflect depressive-like behavior. Three out of 8 monkeys administered IFN-alpha displayed depressive-like huddling behavior, comparable to the 30-50% of patients that develop major depression during IFN-alpha therapy (3-7).

Regarding the mechanism of huddling behavior, several possibilities have been considered. Huddling was first reported in rhesus monkeys following chronic administration of reserpine, a compound known to deplete monoamines including 5-HT, norepinephrine and DA (29). Of relevance to the role of DA in huddling behavior, low doses of DA partial agonists (which can act as DA antagonists) have been found to induce huddling behavior in rhesus monkeys (32). In the current study, concentrations of the DA metabolite, HVA, were reduced by IFN-alpha primarily in monkeys that displayed huddling behavior. Furthermore, HVA concentrations in monkeys that huddled were negatively correlated with time spent huddling during IFN-alpha administration. Of relevance to the impact of IFN-alpha on DA, IFN-alpha treatment of mice has been associated with decreased whole brain homogenate DA concentrations (13). Moreover, DAergic drugs (e.g. levodopa) have been used to treat Parkinson-like movement disorders in patients undergoing IFN-alpha therapy (54). Consequently, the relationship between CSF HVA concentrations and IFN-alpha-induced depressive-like huddling behavior support the notion that DAergic tone may contribute to cytokine-induced behavioral alterations. Finally, the mechanism by which IFN-alpha alters DA metabolism is unclear. Nevertheless, one possibility is that IFN-alpha may influence DA neurotransmission via direct actions on opioid receptors in DA-relevant basal ganglia circuits (19-21). Opioids have been shown to influence dopamine release in ventral striatum through a calcium-dependent mechanism (55).

Another potential pathway involved in huddling behavior is increased central CRF. Marked huddling behavior has been reported in rhesus monkeys following i.c.v. administration of CRF (31), which was also accompanied by reductions in outward directed behaviors (30), similar to the reduction in environmental exploration during IFN-alpha. Consistent with the association between central CRF and huddling behavior, IFN-alpha increased plasma ACTH and cortisol concentrations, and monkeys that expressed depressive-like huddling behavior exhibited a higher mean ACTH response than those that did not. This finding is of interest considering that patients who developed major depression during IFN-alpha therapy for malignant melanoma exhibited exaggerated plasma ACTH and cortisol responses to the first injection of IFN-alpha (8). Of note, despite exhibiting increased ACTH, monkeys that displayed depressive-like huddling behavior exhibited normal (or slightly decreased) cortisol. This dissociation between ACTH and cortisol responses has been reported in women exposed to early life stress who are also at risk for depression and exhibited increased ACTH responses to CRF but blunted cortisol responses, likely due to decreased adrenal sensitivity (56). These data suggest that monkeys who huddled may have neuroendocrine/behavioral responses that are related in part to their previous environmental stress history.

Although peripheral hormone and cytokine responses to IFN-alpha diminished over time especially in dominant monkeys (similar to IFN-alpha-treated patients), persistent behavioral alterations were observed, indicating that central neuroendocrine/neurotransmitter adaptations may occur independently of peripheral responses. Indeed, in a study in humans, it was found

that following three weeks of IFN-alpha administration (a time when no HPA-axis response to IFN-alpha was observed), patients displayed enhanced neuroendocrine responsiveness to CRF as manifested by increased plasma ACTH and cortisol (9). These findings support the notion that neuroendocrine and neurotransmitter adaptations may underlie persistent behavioral alterations, even in the absence of peripheral neuroendocrine responses to IFN-alpha. It should be noted that despite potent activation of the HPA-axis by IFN-alpha, increased CSF CRF concentrations were not detected in this study, possibly due to limitations in assay sensitivity, sampling techniques, or the source of CSF CRF which may not reflect brain regions relevant to the observed behavioral changes.

In terms of the ability of cells from rhesus monkeys to respond to IFN-alpha, experiments validated IFN-alpha receptor expression in IFN-alpha naïve rhesus monkey PBMCs and confirmed that rHu-IFN-alpha can activate relevant IFN-alpha signaling pathways (STAT1) in these animals. To our knowledge, this is the first characterization of the IFNAR1 in rhesus monkeys.

Several limitations in the study design should be noted. First, only 8 animals were examined. Nevertheless, all animals received both saline and IFN-alpha to increase statistical power. Furthermore, although results from small samples sizes should be interpreted with caution, effects sizes for statistically significant results in this study (e.g. anxiety: 0.8, exploratory behavior: 0.935, ACTH: 1.68, IL-6: 2.05, CSF IFN-alpha: 1.68) were robust (57). Second, because the study was an initial characterization of behavioral and biological effects of IFN-alpha in monkeys, a number of parameters (e.g. social interactions, cognitive alterations, diurnal cortisol secretion) were not assessed. Therefore, whether IFN-alpha-induced behavioral and biological changes encompass the entire spectrum of depression (as manifested in rhesus animals or humans) cannot be established. Future studies probing the depressive-like syndrome in monkeys, including expanded behavioral assessments as well as more elaborate sampling protocols evaluating circadian rhythms and the response to stress or other neuroendocrine challenges (e.g. CRF) are warranted. Finally, body temperature was not monitored, and therefore, its contributions to behaviors such as huddling cannot be determined. Nevertheless, the febrile response to repeated IFN-alpha administration is typically brief and diminishes with repeated exposure in humans (58) unlike huddling which persisted throughout the study period.

In summary, rhesus monkeys exhibited IFN-alpha-induced behavioral changes that resemble those exhibited by humans. Furthermore, IFN-alpha stimulated relevant neuroendocrine and immune pathways, and there was preliminary evidence that IFN-alpha-induced depressive-like huddling behavior is mediated by alterations in DA and/or CRF. Finally, these data suggest that rhesus monkeys administered IFN-alpha may provide a valid animal model to investigate depressive effects of cytokines and provide a unique opportunity to explore novel treatment strategies for cytokine-induced behavioral changes.

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References

1. Kirkwood JM, Ernstoff MS. Role of interferons in the therapy of melanoma. J Invest Dermatol 1990;95:180S–184S. [PubMed: 1701805]

2. Saracco G, Olivero A, Ciancio A, Carenzi S, Rizzetto M. Therapy of chronic hepatitis C: a critical review. Curr Drug Targets Infect Disord 2003;3:25–32. [PubMed: 12570730]

3. Hauser P, Khosla J, Aurora H, Laurin J, Kling MA, Hill J, et al. A prospective study of the incidence and open-label treatment of interferon-induced major depressive disorder in patients with hepatitis C. Mol Psychiatry 2002;7:942–947. [PubMed: 12399946]

4. Kraus MR, Schafer A, Faller H, Csef H, Scheurlen M. Psychiatric symptoms in patients with chronic hepatitis C receiving interferon alfa-2b therapy. J Clin Psychiatry 2003;64:708–714. [PubMed: 12823087]

5. Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, Goodkin RS, et al. Paroxetine for the prevention of depression induced by high-dose interferon alfa. N Engl J Med 2001;344:961–966. [PubMed: 11274622]

6. Pariante CM, Orru MG, Baita A, Farci MG, Carpiniello B. Treatment with interferon-alpha in patients with chronic hepatitis and mood or anxiety disorders. Lancet 1999;354:131–132. [PubMed: 10408496]

7. Raison CL, Borisov AS, Broadwell SD, Capuron L, Woolwine BJ, Jacobson IM, et al. Depression during pegylated interferon-alpha plus ribavirin therapy: prevalence and prediction. J Clin Psychiatry 2005;66:41–48. [PubMed: 15669887]

8. Capuron L, Raison CL, Musselman DL, Lawson DH, Nemeroff CB, Miller AH. Association of exaggerated HPA axis response to the initial injection of interferon-alpha with development of depression during interferon-alpha therapy. Am J Psychiatry 2003;160:1342–1345. [PubMed: 12832253]

9. Gisslinger H, Svoboda T, Clodi M, Gilly B, Ludwig H, Havelec L, et al. Interferon-alpha stimulates the hypothalamic-pituitary-adrenal axis in vivo and in vitro. Neuroendocrinol 1993;57:489–495.

10. Raber J, Koob GF, Bloom FE. Interferon-alpha and transforming growth factor-beta 1 regulate corticotropin-releasing factor release from the amygdala: comparison with the hypothalamic response. Neurochem Int 1997;30:455–463. [PubMed: 9106261]

11. Shimizu H, Ohtani K, Sato N, Nagamine T, Mori M. Increase in serum interleukin-6, plasma ACTH and serum cortisol levels after systemic interferon-alpha administration. Endocr J 1995;42:551–556. [PubMed: 8556063]

12. Kumai T, Tateishi T, Tanaka M, Watanabe M, Shimizu H, Kobayashi S. Effect of interferon-alpha on tyrosine hydroxylase and catecholamine levels in the brain of rats. Life Sci 2000;67:663–669. [PubMed: 12659172]

13. Shuto H, Kataoka Y, Horikawa T, Fujihara N, Oishi R. Repeated interferon-alpha administration inhibits dopaminergic neural activity in the mouse brain. Brain Res 1997;747:348–351. [PubMed: 9046014]

14. Kamata M, Higuchi H, Yoshimoto M, Yoshida K, Shimizu T. Effect of single intracerebroventricular injection of alpha-interferon on monoamine concentrations in the rat brain. Eur Neuropsychopharmacol 2000;10:129–132. [PubMed: 10706995]

15. Kitagami T, Yamada K, Miura H, Hashimoto R, Nabeshima T, Ohta T. Mechanism of systemically injected interferon-alpha impeding monoamine biosynthesis in rats: role of nitric oxide as a signal crossing the blood-brain barrier. Brain Res 2003;978:104–114. [PubMed: 12834904]

16. Capuron L, Neurauter G, Musselman DL, Lawson DH, Nemeroff CB, Fuchs D, et al. Interferon-alpha-induced changes in tryptophan metabolism. relationship to depression and paroxetine treatment. Biol Psychiatry 2003;54:906–914. [PubMed: 14573318]

17. Zhu CB, Blakely RD, Hewlett WA. The proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha activate serotonin transporters. Neuropsychopharmacol 2006;31:2121–2131.

18. Sanchez MM, Alagbe O, Felger JC, Zhang J, Graff AE, Grand AP, et al. Activated p38 MAPK is Associated with Decreased CSF 5-HIAA and Increased Maternal Rejection During Infancy in Young Adult Rhesus Monkeys. Mol Psychiatry. 2007in press

19. Ho BT, Huo YY, Lu JG, Tansey LW, Levin VA. Opioid-dopaminergic mechanisms in the potentiation of d-amphetamine discrimination by interferon-alpha. Pharmacol Biochem Behav 1992;42:57–60. [PubMed: 1528947]

20. Schaefer M, Schwaiger M, Pich M, Lieb K, Heinz A. Neurotransmitter changes by interferon-alpha and therapeutic implications. Pharmacopsychiatry 2003;36(Suppl 3):S203–206. [PubMed: 14677080]

21. Wang JY, Zeng XY, Fan GX, Yuan YK, Tang JS. mu- but not delta- and kappa-opioid receptor mediates the nucleus submedius interferon-alpha-evoked antinociception in the rat. Neurosci Lett 2006;397:254–258. [PubMed: 16406668]
22. Reite M, Laudenslager M, Jones J, Crnic L, Kaemingk K. Interferon decreases REM latency. Biol Psychiatry 1987;22:104–107. [PubMed: 3790631]
23. Capuron L, Gumnick JF, Musselman DL, Lawson DH, Reemsnyder A, Nemeroff CB, et al. Neurobehavioral effects of interferon-alpha in cancer patients: phenomenology and paroxetine responsiveness of symptom dimensions. Neuropsychopharmacol 2002;26:643–652.
24. Altmann SA. A field study of the sociobiology of rhesus monkeys, Macaca mulatta. Ann N Y Acad Sci 1962;102:338–435. [PubMed: 14012344]
25. Kalin NH. Nonhuman primate studies of fear, anxiety, and temperament and the role of benzodiazepine receptors and GABA systems. J Clin Psychiatry 2003;64(Suppl 3):41–44. [PubMed: 12662133]
26. Schino G, Perretta G, Taglioni AM, Monaco V, Troisi A. Primate displacement activities as an ethopharmacological model of anxiety. Anxiety 1996;2:186–191. [PubMed: 9160621]
27. Troisi A. Displacement activities as a behavioral measure of stress in nonhuman primates and human subjects. Stress 2002;5:47–54. [PubMed: 12171766]
28. Troisi A, Schino G, D'Antoni M, Pandolfi N, Aureli F, D'Amato FR. Scratching as a behavioral index of anxiety in macaque mothers. Behav Neural Biol 1991;56:307–313. [PubMed: 1759948]
29. McKinney WT Jr, Eising RG, Moran EC, Suomi SJ, Harlow HF. Effects of reserpine on the social behavior of rhesus monkeys. Dis Nerv Syst 1971;32:735–741. [PubMed: 5002259]
30. Strome EM, Wheler GH, Higley JD, Loriaux DL, Suomi SJ, Doudet DJ. Intracerebroventricular corticotropin-releasing factor increases limbic glucose metabolism and has social context-dependent behavioral effects in nonhuman primates. Proc Natl Acad Sci U S A 2002;99:15749–15754. [PubMed: 12438692]
31. Kalin NH. Behavioral effects of ovine corticotropin-releasing factor administered to rhesus monkeys. Fed Proc 1985;44:249–253. [PubMed: 3871411]
32. Rosenzweig-Lipson S, Hesterberg P, Bergman J. Observational studies of dopamine D1 and D2 agonists in squirrel monkeys. Psychopharmacol (Berl) 1994;116:9–18.
33. Maestripieri D, Higley JD, Lindell SG, Newman TK, McCormack KM, Sanchez MM. Early maternal rejection affects the development of monoaminergic systems and adult abusive parenting in rhesus macaques (Macaca mulatta). Behav Neurosci 2006;120:1017–1024. [PubMed: 17014253]
34. Maestripieri D, McCormack K, Lindell SG, Higley JD, Sanchez MM. Influence of parenting style on the offspring's behaviour and CSF monoamine metabolite levels in crossfostered and noncrossfostered female rhesus macaques. Behav Brain Res 2006;175:90–95. [PubMed: 16971003]
35. Broadbear JH, Winger G, Rivier JE, Rice KC, Woods JH. Corticotropin-releasing hormone antagonists, astressin B and antalarmin: differing profiles of activity in rhesus monkeys. Neuropsychopharmacol 2004;29:1112–1121.
36. Kalin NH, Shelton SE, Davidson RJ. The role of the central nucleus of the amygdala in mediating fear and anxiety in the primate. J Neurosci 2004;24:5506–5515. [PubMed: 15201323]
37. Reyes TM, Coe CL. The proinflammatory cytokine network: interactions in the CNS and blood of rhesus monkeys. Am J Physiol 1998;274:R139–144. [PubMed: 9458910]
38. Willette AA, Lubach GR, Coe CL. Environmental context differentially affects behavioral, leukocyte, cortisol, and interleukin-6 responses to low doses of endotoxin in the rhesus monkey. Brain Behav Immun. 2007
39. Xiao E, Xia-Zhang L, Vulliemoz NR, Ferin M, Wardlaw SL. Leptin modulates inflammatory cytokine and neuroendocrine responses to endotoxin in the primate. Endocrinol 2003;144:4350–4353.
40. Villinger F, Brar SS, Mayne A, Chikkala N, Ansari AA. Comparative sequence analysis of cytokine genes from human and nonhuman primates. J Immunol 1995;155:3946–3954. [PubMed: 7561102]
41. Wightman RM, Plotsky PM, Strope E, Delcore R Jr, Adams RN. Liquid chromatographic monitoring of CSF metabolites. Brain Res 1977;131:345–349. [PubMed: 890461]
42. Kalin NH, Shelton SE, Barksdale CM, Brownfield MS. A diurnal rhythm in cerebrospinal fluid corticotrophin-releasing hormone different from the rhythm of pituitary-adrenal activity. Brain Res 1987;426:385–391. [PubMed: 2825918]

43. Song C, Horrobin DF, Leonard BE. The comparison of changes in behavior, neurochemistry, endocrine, and immune functions after different routes, doses and durations of administrations of IL-1beta in rats. Pharmacopsychiatry 2006;39:88–99. [PubMed: 16721697]
44. Anisman H, Merali Z. Anhedonic and anxiogenic effects of cytokine exposure. Adv Exp Med Biol 1999;461:199–233. [PubMed: 10442175]
45. Makino M, Kitano Y, Hirohashi M, Takasuna K. Enhancement of immobility in mouse forced swimming test by treatment with human interferon. Eur J Pharmacol 1998;356:1–7. [PubMed: 9761417]
46. Sammut S, Goodall G, Muscat R. Acute interferon-alpha administration modulates sucrose consumption in the rat. Psychoneuroendocrinol 2001;26:261–272.
47. Zalcman S, Murray L, Dyck DG, Greenberg AH, Nance DM. Interleukin-2 and -6 induce behavioral-activating effects in mice. Brain Res 1998;811:111–121. [PubMed: 9804916]
48. Dantzer R, Wollman EE, Yirmiya R. Cytokines and depression: an update. Brain Behav Immun 2002;16:501–502. [PubMed: 12401463]
49. Collins JM, Riccardi R, Trown P, O'Neill D, Poplack DG. Plasma and cerebrospinal fluid pharmacokinetics of recombinant interferon alpha A in monkeys: comparison of intravenous, intramuscular, and intraventricular delivery. Cancer Drug Deliv 1985;2:247–253. [PubMed: 4063949]
50. Greig NH, Soncrant TT, Wozniak KM, Rapoport SI. Plasma and tissue pharmacokinetics of human interferon-alpha in the rat after its intravenous administration. J Pharmacol Exp Ther 1988;245:574–580. [PubMed: 2835475]
51. Cassidy EM, Manning D, Byrne S, Bolger E, Murray F, Sharifi N, et al. Acute effects of low-dose interferon-alpha on serum cortisol and plasma interleukin-6. J Psychopharmacol 2002;16:230–234. [PubMed: 12236630]
52. Sissolak G, Hoffbrand AV, Mehta AB, Ganeshaguru K. Effects of interferon-alpha (IFN) on the expression of interleukin 1-beta (IL-1), interleukin 6 (IL-6), granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor-alpha (TNF) in acute myeloid leukemia (AML) blasts. Leukemia 1992;6:1155–1160. [PubMed: 1434798]
53. Taylor JL, Grossberg SE. The effects of interferon-alpha on the production and action of other cytokines. Semin Oncol 1998;25:23–29. [PubMed: 9482537]
54. Sunami M, Nishikawa T, Yorogi A, Shimoda M. Intravenous administration of levodopa ameliorated a refractory akathisia case induced by interferon-alpha. Clin Neuropharmacol 2000;23:59–61. [PubMed: 10682234]
55. McGinty JF. Regulation of neurotransmitter interactions in the ventral striatum. Ann N Y Acad Sci 1999;877:129–139. [PubMed: 10415647]
56. Heim C, Newport DJ, Bonsall R, Miller AH, Nemeroff CB. Altered pituitary-adrenal axis responses to provocative challenge tests in adult survivors of childhood abuse. Am J Psychiatry 2001;158:575–581. [PubMed: 11282691]
57. Cohen, J. Statistical power analysis for the behavioral sciences. 2. Hillsdale, NJ: Erlbaum; 1988.
58. Breinig MC, Ho M, White L, Armstrong JA, Pazin GJ, Haverkos HW, et al. Effect of prolonged administration of interferon-alpha on pharmacokinetics, fever, lymphocyte proliferative response, and NK cell activity. J Interferon Res 1982;2:195–207. [PubMed: 7119506]

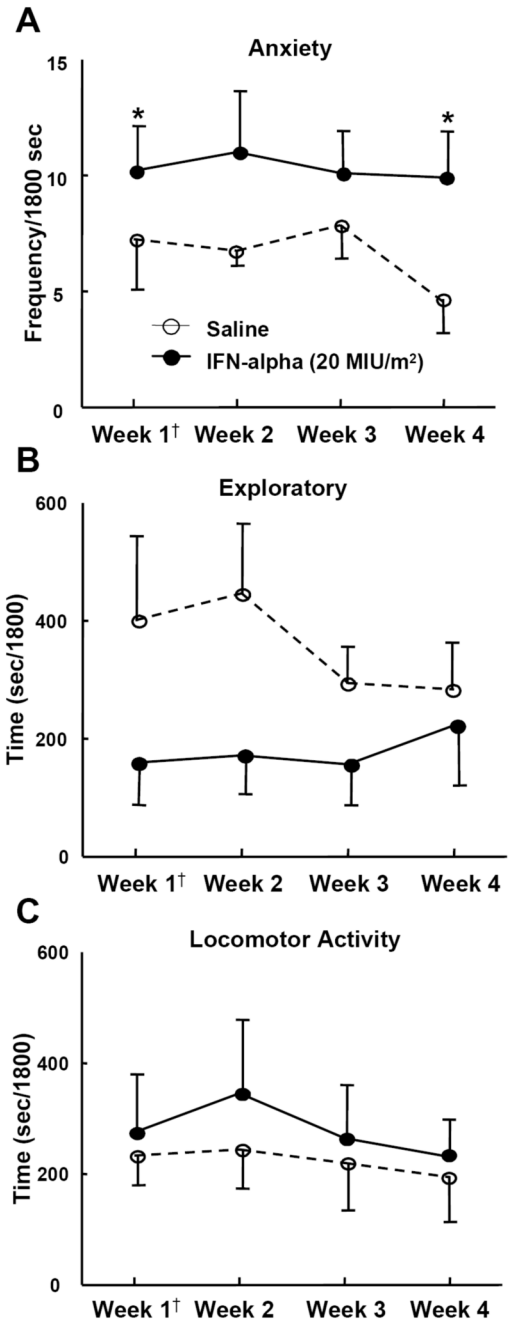

Figure 1.

Anxiety-like behavior, exploratory behavior, and locomotor activity during IFN-alpha or saline administration. IFN-alpha (20 MIU/m^2) or saline was administered s.c. to 8 rhesus monkeys for 4 weeks in counterbalanced fashion, and behavior was assessed weekly by videotape at 2 hours post-injection. Compared to saline, IFN-alpha increased anxiety-like behaviors (**A**), decreased exploratory behaviors (**B**), and had no significant effect on locomotor activity (**C**). Data are presented as the mean (+/- SEM) at each time point. * - indicates a significant difference between treatment groups at indicated time points as revealed by post-hoc analyses ($p<0.05$). † - indicates the initiation of saline or IFN-alpha administration (day 1).

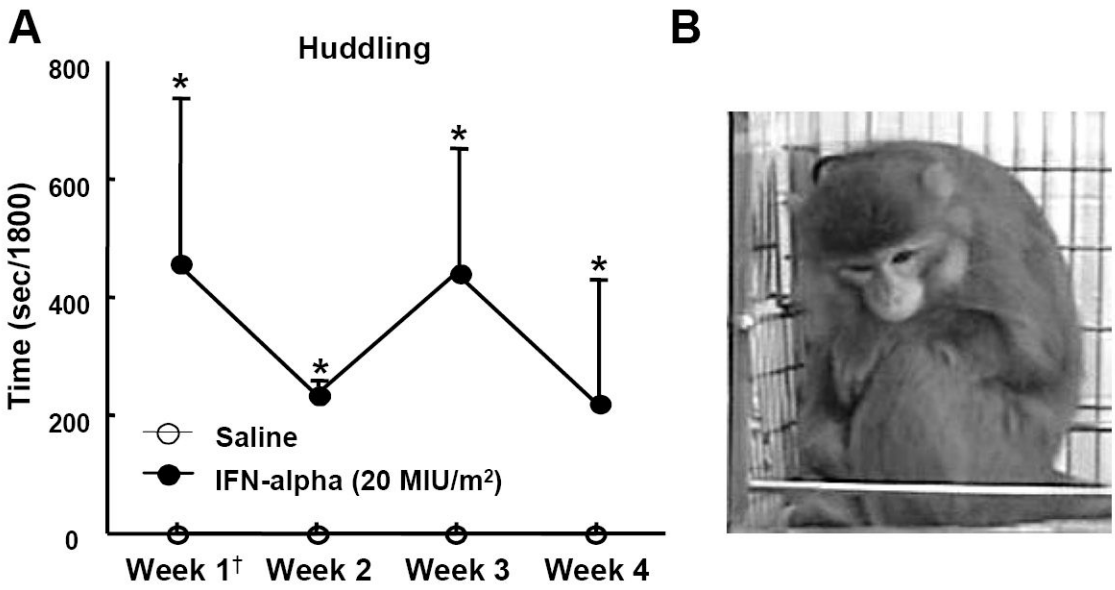

Figure 2.

Depressive-like huddling behavior during IFN-alpha or saline administration. IFN-alpha (20 MIU/m^2) or saline was administered s.c. for 4 weeks to 8 rhesus monkeys in counterbalanced fashion, and behavior was assessed weekly by videotape at 2 hours post-injection. Administration of IFN-alpha was associated with persistent huddling behavior in 3 animals (**A**). A photograph of a monkey huddling during IFN-alpha administration appears in **B**. Data are presented as the mean (+/- SEM) for these 3 animals at each time point during either saline or IFN-alpha treatment. * - indicates a significant difference between treatment groups at indicated time points as revealed by post-hoc analyses ($p<0.05$). † - indicates the initiation of saline or IFN-alpha administration (day 1).

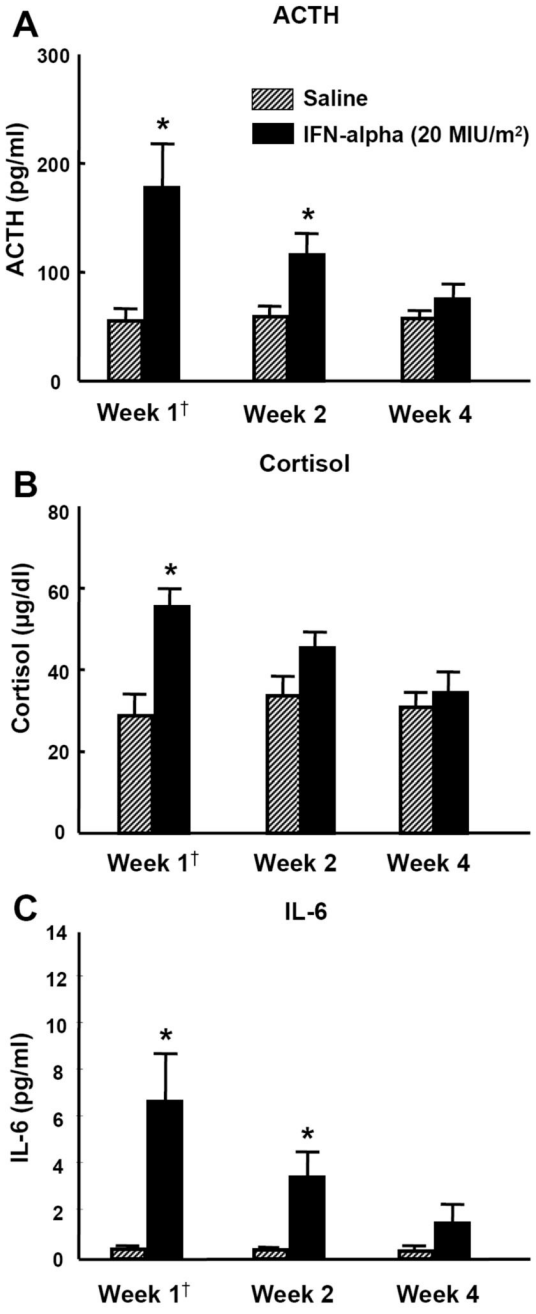

Figure 3.

Plasma ACTH, cortisol and IL-6 concentrations during IFN-alpha or saline administration. IFN-alpha (20 MIU/m^2) or saline was administered s.c. to 8 rhesus monkeys for 4 weeks in counterbalanced fashion, and plasma was collected in weeks 1,2 and 4, 3 hours post-injection, for the analyses of plasma hormones and cytokines. Compared to saline, IFN-alpha induced significant increases in plasma ACTH (**A**), cortisol (**B**), and IL-6 (**C**) concentrations, which were attenuated over time. Data are presented as the mean (+/- SEM) at each time point. * - indicates a significant difference between treatment groups at indicated time points as revealed by post-hoc analyses ($p<0.05$). † - indicates the initiation of saline or IFN-alpha administration (day 1).

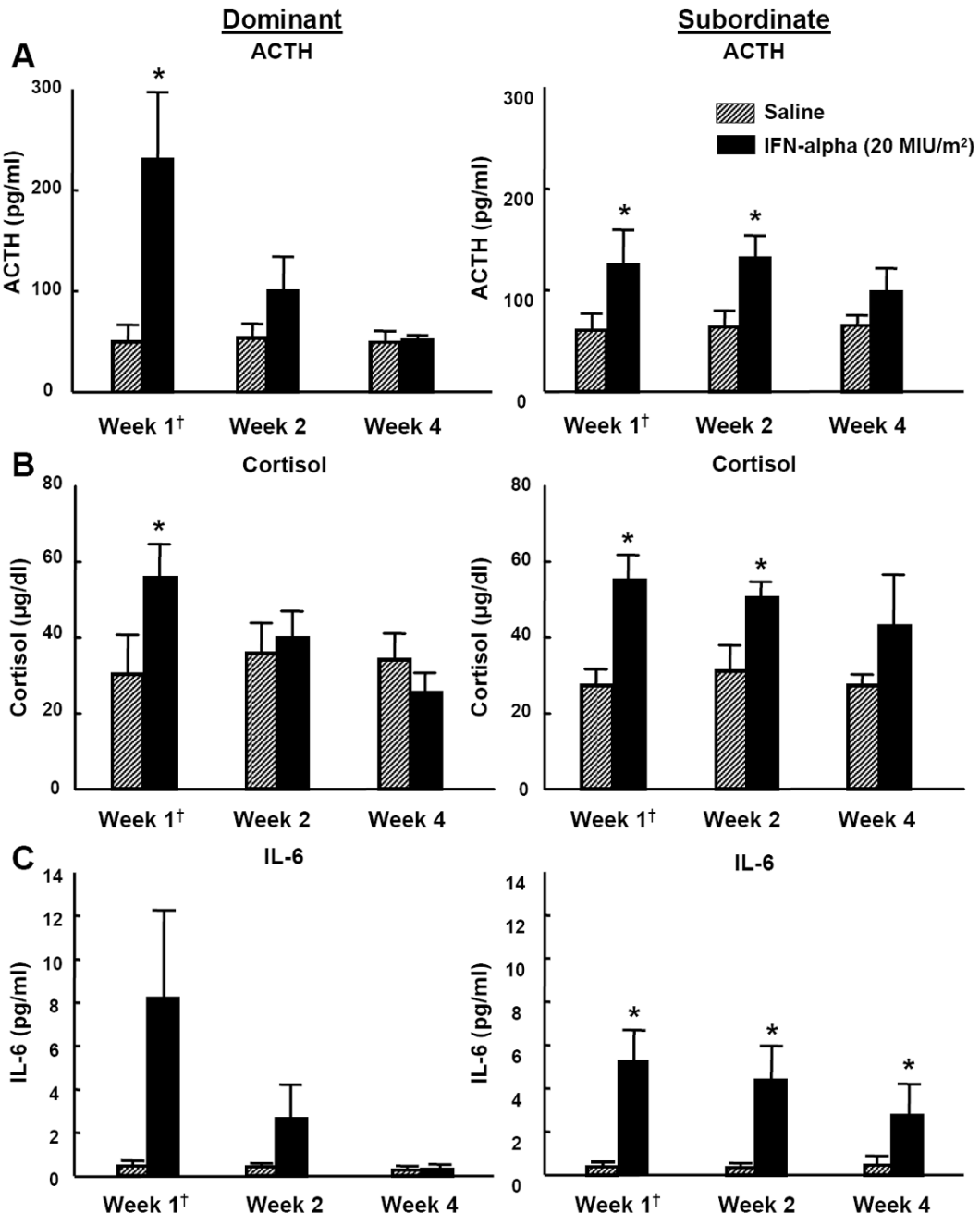

Figure 4.

Plasma ACTH, cortisol and IL-6 concentrations during IFN-alpha or saline administration in dominant and subordinate monkeys. IFN-alpha (20 MIU/m^2) or saline was administered s.c. for 4 weeks to 8 rhesus monkeys in counterbalanced fashion, and plasma was collected in weeks 1,2 and 4, 3 hours post-injection, for the analyses of hormones and cytokines. Compared to dominant monkeys (n=4) who had significant elevations in ACTH (**A**) and cortisol (**B**) at week 1 only, subordinate animals (n=4) had more persistent elevations in plasma ACTH (**A**), cortisol (**B**), and IL-6 (**C**) with increases in ACTH and cortisol at weeks 1 and 2, and IL-6 at weeks 1-3. Data are presented as the mean (+/- SEM) at each time point. * - indicates a significant difference between treatment groups at indicated time points as revealed by post-

hoc analyses ($p<0.05$). † - indicates the initiation of saline or IFN-alpha administration (day 1).

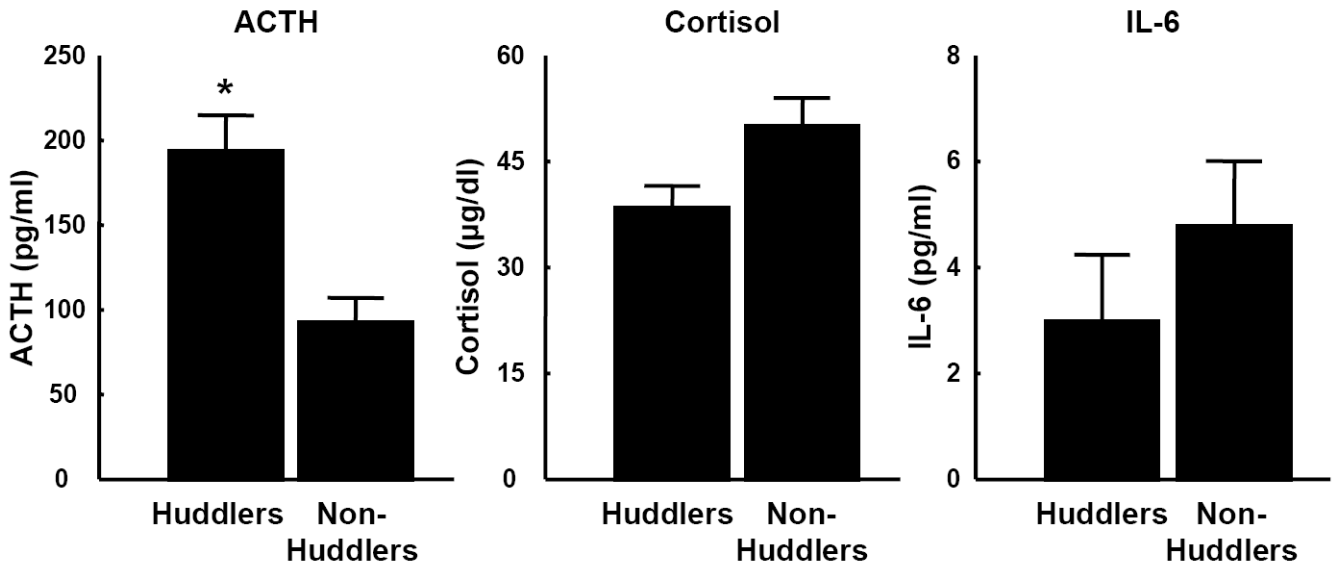

Figure 5.

Plasma ACTH, cortisol and IL-6 responses to IFN-alpha in monkeys that displayed depressive-like huddling behavior. IFN-alpha (20 MIU/m^2) or saline was administered s.c. for 4 weeks to 8 rhesus monkeys in counterbalanced fashion, and plasma was collected in weeks 1,2 and 4, 3 hours post-injection, for the analyses of hormones and cytokines. Behavior was assessed weekly by videotape 2 hours post-injection. Monkeys that huddled ("Huddlers", n=3) demonstrated significantly higher average plasma ACTH concentrations during the study than those that did not ("Non-Huddlers", n=5). Data are represented as mean (+/- SEM). * - indicates a significant difference between groups $p<0.05$.

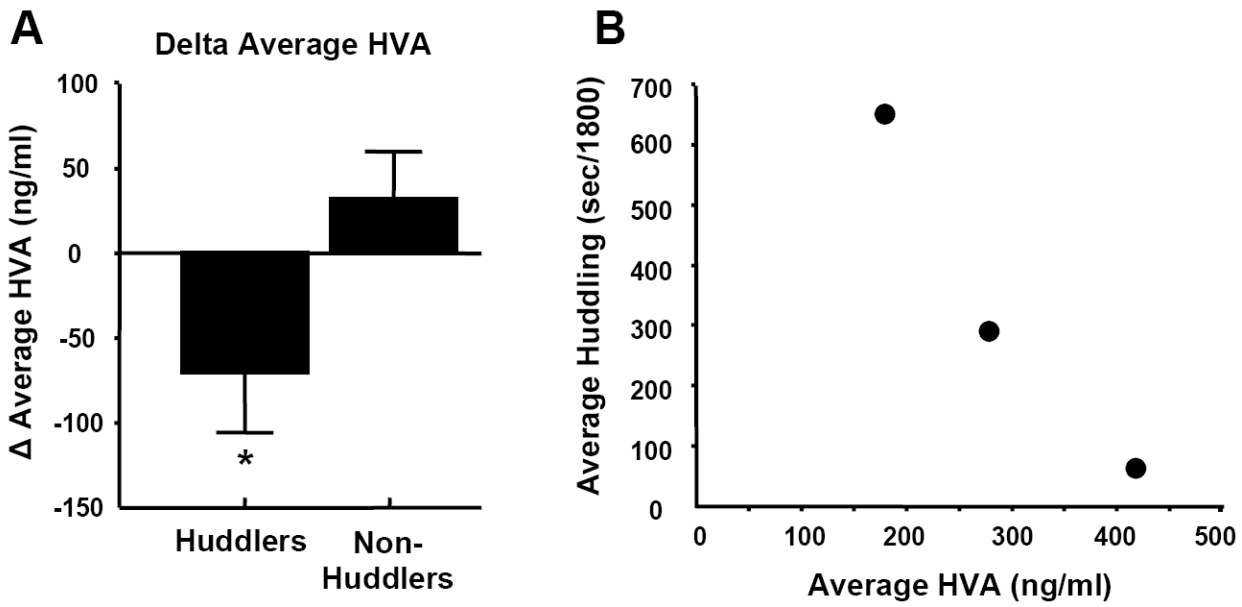

Figure 6.

Depressive-like huddling behavior was associated with CSF HVA concentrations. IFN-alpha (20 MIU/m^2) or saline was administered s.c. for 4 weeks to 8 rhesus monkeys in counterbalanced fashion, and CSF was collected from the cisterna magna at weeks 1, 2 and 4, 3 hours post-injection, for the analyses of the monoamine metabolites HVA, DOPAC, and 5-HIAA. Behavior was assessed weekly by videotape 2 hours post-injection. IFN-alpha significantly reduced average CSF HVA concentrations during the study compared to saline values in monkeys who displayed huddling behavior ("Huddlers", n=3) in contrast to those that did not ("Non-Huddlers", n=5). Delta (IFN-alpha minus saline) values are presented as mean (+/- SEM) (**A**). The average amount of time spent huddling during the study correlated with average CSF HVA concentrations during IFN-alpha administration (**B**). * - indicates a significant difference between groups ($p<0.05$).

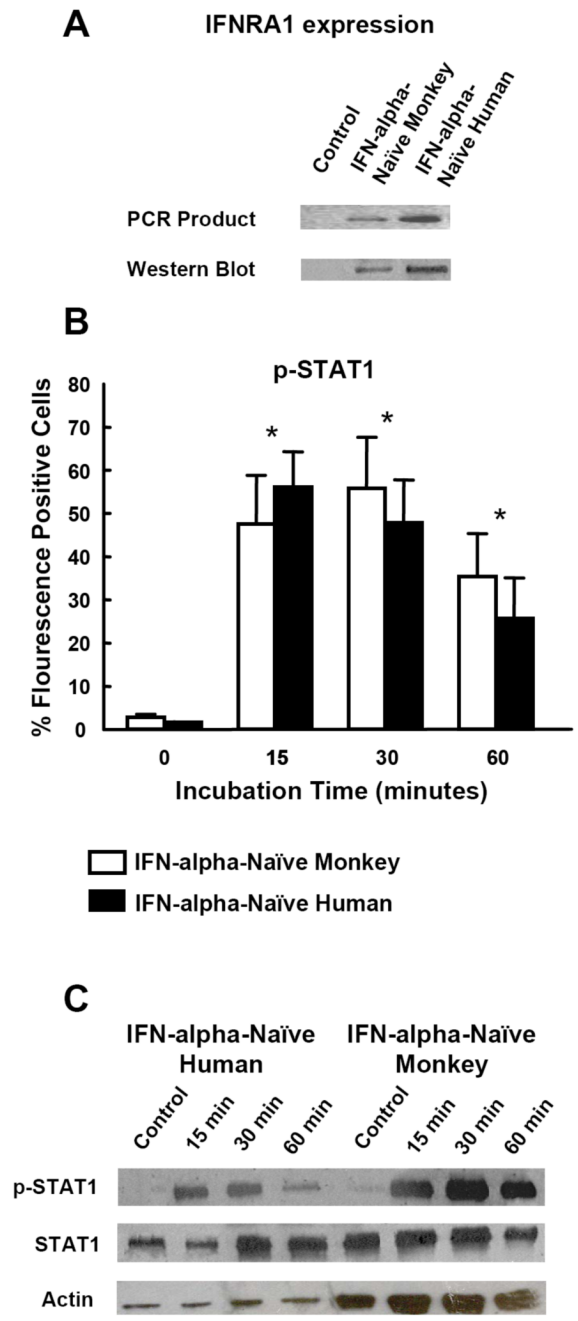

Figure 7.

Expression and activation of IFNAR1 in rhesus monkey PBMCs. IFNAR1 was expressed comparably in IFN-alpha naïve rhesus monkey and human PBMCs as detected by RT-PCR and Western blot. Control samples included a mouse cell line (HT22) for Western blot and a no-RT sample for RT-PCR. (**A**). Incubation of PBMCs from IFN-alpha-naïve rhesus monkeys and humans with rHu-IFN-alpha-A (5000 IU/ml) for 15,30, and 60 minutes activated pSTAT-1 expression to a similar degree as detected by flow cytometry (**B**) and Western blot (**C**).

Table 1

Average behavior in rhesus monkeys during administration of IFN-alpha or saline.

		All Monkeys (N=8)	Dominant (n=4)	Subordinate (n=4)
Anxiety (frequency)	Saline	6.4 (1.5)	7.5 (3.2)	5.4 (0.7)
	IFN-alpha	10.3 (1.9)*	11.2 (1.9)	9.3 (3.6)
Exploratory (sec/1800)	Saline	370.9 (72.2)	441.5 (170.6)	300.3 (44.7)
	IFN-alpha	181.8 (70.8)*	221.7 (95.3)	141.8 (34.5)
Locomotor (sec/1800)	Saline	223.2 (72.4)	313.5 (136.6)	132.9 (18.5)
	IFN-alpha	279.1 (92.5)	115.3 (35.1)	442.9 (144.3)+
Huddling (sec/1800)	Saline†	0.0 (0.0)	N/A	N/A
	IFN-alpha†	338.9 (169.3)	N/A	N/A

Data are presented as mean (+/- SEM) values of each variable averaged across 4 time points for each treatment group.

* $p<0.05$ - IFN-alpha compared to saline

+ $p<0.05$ - subordinate compared to dominant

† n=3 per group, N/A = no comparisons made when n < 3.

Table 2

Average plasma hormone and cytokine concentrations in rhesus monkeys during administration of IFN-alpha or saline.

		All Monkeys (N=8)	Dominant (n=4)	Subordinate (n=4)
ACTH (pg/ml)	Saline	57.1 (9.3)	49.5 (13.9)	63.0 (12.2)
	IFN-alpha	122.9 (17.3)**	140.9 (34.8)*	111.7 (24.5)
Cortisol (µg/dl)	Saline	30.8 (4.2)	33.5 (16.2)	28.5 (6.4)
	IFN-alpha	45.7 (3.3)*	40.5 (12.5)	49.6 (7.3)*
IL-6 (pg/ml)	Saline	0.4 (0.1)	0.4 (0.1)	0.4 (0.3)
	IFN-alpha	4.1 (0.9)**	3.7 (1.8)*	4.1 (0.7)*
IL-1beta (fg/ml)	Saline	115.5 (29.2)	77.1 (43.0)	153.9 (28.6)
	IFN-alpha	217.6 (81.3)	157.6 (48.2)	277.6 (11.6)
IFN-alpha (pg/ml)	Saline	7.6 (1.5)	7.0 (2.8)	7.0 (2.1)
	IFN-alpha	1259.1(359.1)*	1201.6 (442.6)	1316.7 (635.3)*

Data are presented as mean (+/- SEM) values of each variable averaged across 3 time points for each treatment group.

* $p<0.05$

** $p<0.01$ - IFN-alpha compared to saline.

Table 3

Average CSF neurotransmitter, neuropeptide, and cytokine concentrations in rhesus monkeys during administration of IFN-alpha or saline.

		All Monkeys (N=8)	Dominant (n=4)	Subordinate (n=4)
HVA (ng/ml)	Saline	323.3 (28.6)	320.4 (53.6)	326.3 (30.6)
	IFN-alpha	309.9 (27.3)	313.5 (54.3)	306.3 (22.9)
DOPAC (ng/ml)	Saline	4.5 (0.9)	5.2 (1.8)	3.8 (0.5)
	IFN-alpha	4.6 (0.6)	5.3 (1.1)	4.0 (0.4)
5-HIAA (ng/ml)	Saline	76.3 (7.2)	79.7 (14.4)	72.9 (4.9)
	IFN-alpha	76.5 (6.2)	81.4 (11.5)	71.6 (5.6)
CRF (pg/ml)	Saline	165.8 (31.5)	121.3 (23.3)	225.2 (52.6)
	IFN-alpha	155.7 (12.8)	138.1 (6.7)	168.9 (19.2)
IL-6 (pg/ml)	Saline	3.5 (0.7)	3.2 (0.9)	4.0 (1.4)
	IFN-alpha	4.2 (0.6)	4.7 (0.9)	3.6 (0.9)
IL-1beta (fg/ml)	Saline†	281.1 (34.1)	N/A	N/A
	IFN-alpha†	388.7 (37.3)*	N/A	N/A
IFN-alpha (pg/ml)	Saline†	38.8 (17.1)	N/A	N/A
	IFN-alpha†	107.7 (23.4)*	N/A	N/A

Data are presented as mean (+/- SEM) values of each variable averaged across 3 time points for each treatment group.

*p<0.05 - IFN-alpha compared to saline

†n=4 per group, N/A = no comparisons made when n < 3.