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Individual Differences in Prefrontal Activation Asymmetry Predict Natural Killer Cell Activity at Rest and in Response to Challenge

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Reliable individual differences in electrophysiological measures of prefrontal activation asymmetry exist and predict dispositional mood and other psychological and biological indices of affective style. Subjects with greater relative right-sided activation report more dispositional negative affect and react with greater intensity to negative emotional challenges than their left-activated counterparts. We previously established that such individual differences in measures of prefrontal activation asymmetry were related to basal NK function, with left-activated subjects exhibiting higher levels of NK function than right-activated subjects. The present study was designed to replicate and extend these earlier findings. Subjects were tested in five experimental sessions over the course of 1 year. During the first two sessions, baseline measures of brain electrical activity were obtained to derive indices of asymmetric activation. During sessions 3 and 4, blood samples were taken during a nonstressful period in the semester and then 24 h prior to the subjects' most important final examination. During session 5, subjects were presented with positive and negative film clips 30 min in duration. Blood samples were obtained before and after the film clips. Subjects with greater relative right-sided activation at baseline showed lower levels of basal NK function. They also showed a greater decrease in NK function during the final exam period compared to the baseline period. Subjects with greater relative left-sided activation showed a larger increase in NK function from before to after the positive film clip. These findings indicate that individual differences in electrophysiological measures of asymmetric prefrontal activation account for a significant portion of variance in both basal levels of, and change in NK function. © 1999 Academic Press

A salient characteristic of responses to emotional challenges and stressful life events is the variability among individuals in the psychological and biological changes that are produced. The same event can produce vastly different responses across different individuals (see Davidson, 1998a,b, for review). Some investigators have suggested that individual differences in parameters of emotional reactivity or affective style can influence immune function and health status in response to infectious challenge (e.g., Cohen, Tyrell, & Smith, 1993).

A number of different approaches have also been used to evaluate individual differences in immunity and the susceptibility to immune-related disease. Cohen and his colleagues, for example, found that subjective emotional distress (perceived stress and negative affect) was associated with the likelihood of infection and greater cold symptomatology following a controlled exposure to rhinoviruses (Cohen et al., 1993). Others have also tried to use psychological factors to predict individual variation in the magnitude of immune changes following stressful events. In several studies, low social support and a perceived sense of ''loneliness'' were associated with a greater decrement in natural killer (NK) cell activity in response to the stress of school ex-

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amination week (Glaser, Kiecolt-Glaser, Speicher, & Holliday, 1985; Kang, Coe, Karaszewski, & McCarthy, 1998; Kiecolt-Glaser, Garner, Speicher, Penn, Holliday, & Glaser, 1984). Although many studies have suggested that individual variation in life style, including sleep and dietary patterns or the level of neuroendocrine activation, should also be predictive of the immune responses to this type of stressful life event, it has proven to be difficult to more specifically account for the differential impact of exams across students. The present study utilized a unique approach, which offers a novel and integrated model of brain, emotion, and physiological reactivity, to discern whether we could account for variation in NK responses to exam stress and subsequently to acute emotional stimulation.

Over the past decade, Davidson and his colleagues have been examining individual differences in asymmetric prefrontal activation and their relation to psychological and biological indices of emotional reactivity (Davidson, 1998a,b). Using measures of scalp-recorded brain electrical activity, they established that indices of prefrontal activation asymmetry are stable over time and show excellent internal consistency reliability, thus fulfilling the psychometric requirements of a trait-like construct (Tomarken, Davidson, Wheeler, & Kinney, 1992a). A large corpus of evidence has established the construct validity of individual differences in prefrontal activation asymmetry. Some of the noteworthy findings to date include the following: subjects with greater relative left prefrontal activation report more positive and less negative dispositional mood (Tomarken, Davidson, Wheeler, & Doss, 1992b) and more behavioral activation relative to behavioral inhibition (Sutton & Davidson, 1977) than their right frontally activated counterparts; right frontally activated subjects respond more intensely to negative affective challenges and less intensely to positive affective challenges than their left frontally activated counterparts (Wheeler, Davidson, & Tomarken, 1993); acutely depressed patients (Henriques & Davidson, 1991) as well as remitted depressed patients (Henriques & Davidson, 1990) exhibit decreased left prefrontal activation compared with never-depressed controls; behaviorally inhibited toddlers and young children show greater relative right-sided prefrontal activation than their outgoing, sociable counterparts (Davidson & Rickman, in press). We have also extended the human work to nonhuman primates (Davidson, Kalin, & Shelton, 1993) and have demonstrated that measures of prefrontal activation asymmetry derived from scalp-recorded brain electrical activity are stable over time and that those animals with greater relative right-sided prefrontal activation show higher basal levels of cortisol (Kalin, Larson, Shelton, & Davidson, 1998) than animals who exhibit left prefrontal activation.

Previous research in humans and animals has indicated not only that activity of certain brain regions is associated with immunity, but that there may be an important relation with cerebral asymmetry and hemispheric activation. In a series of pioneering studies and theoretical syntheses, Geschwind and colleagues (Geschwind & Galaburda, 1985) examined relations between handedness, which reflects hemispheric specialization, and autoimmune disease. However, a number of investigators have highlighted the distinction between hemispheric specialization and hemispheric activation and have noted that variations in the latter are superimposed on the former. Thus, an individual may be right-handed and have speech localized in the left hemisphere but show tonic activation of certain right hemisphere regions.

More direct evidence for the existence of an association between cerebral asymmetry and immunity has been provided by animal studies in which effects of unilateral

cortical lesions on measures of immune function have been examined. For example, when comparable areas of either the left or right hemispheres were ablated in mice, different patterns of immune alteration were observed (Barneoud, Neveu, Vitiello, & LeMoal, 1987; Neveu, 1991). Damage to the left side, creating a state of relative right-sided dominance, often elicited more negative effects on splenic lymphocyte proliferation and antibody responses (see review by Renoux & Biziere, 1991). These findings appeared to concur with the finding of greater catecholamine release in rats (Robinson & Coyle, 1980) and reports of greater dysphoria in human patients who had experienced a unilateral stroke in the left hemisphere compared to the right hemisphere (Robinson & Downhill, 1995). More recently, it was found that unilateral hemispheric damage due to either a perinatal insult (cerebral palsy) or an adult stroke resulted in lymphocytes that were less responsive to stimulation with interleukin-2 (IL-2) and this insensitivity was associated with high circulating levels of the soluble IL-2 receptor (Rogers, Coe, & Karaszewski, 1998).

The following study extends this type of brain-immune inquiry to consider the relevance to healthy individuals. Several years ago, we reported on an association between prefrontal brain electrical asymmetry and the level of NK activity in healthy college students (Kang, Davidson, Coe, Wheeler, Tomarken, & Ershler, 1991). These female students had been selected for being extremes on the continuum of right and left frontal activation asymmetry. Our data confirmed our prediction and indicated that right frontally activated individuals had lower levels of NK function than left-activated subjects. The current study tests the applicability of these findings to a normal unselected sample of healthy male and female students.

The present study was an attempt to replicate and extend this earlier work. In our previous study examining relations between immune function and asymmetry, we selected extreme groups based upon electrophysiological measures of prefrontal activation asymmetry and compared them on measures of NK function. In the current study, we sought to determine whether such a relation could be found among unselected individuals. Moreover, we were also interested in whether baseline measures of prefrontal activation asymmetry predicted the magnitude of change in NK function in response to both naturally occurring and experimentally produced emotional challenge. The naturally occurring challenge that we used was the stress of final exams. The experimental laboratory challenge was a positive and negative emotional film clip. We hypothesized that subjects with greater relative right-sided frontal activation would show lower levels of basal NK function. This would confirm our previous finding in an unselected group. We also hypothesized that subjects with increased relative right-sided prefrontal activation would show larger decreases in NK function in response to the stress of final examination. Finally, we predicted that subjects with increased relative right-sided activation would show a larger magnitude reduction in NK function following a negative film clip and that subjects with greater relative left-sided prefrontal activation would show a larger magnitude increase in NK function following a positive film clip.

METHODS

Subjects

Subjects were 24 right-handed undergraduates recruited from the Introduction to Psychology Subject Pool at the University of Wisconsin-Madison. Subjects were be-

tween the ages of 17 and 21 years (average of 18.5 years). Of the 24 subjects, 9 were female. Subjects reported no known neurological or psychological dysfunction. Handedness was assessed by the Chapman Handedness Inventory (Chapman & Chapman, 1987); only subjects reporting exclusive right hand use for at least 10 of 13 tasks were admitted to this study.

Procedures

Subjects participated in five experimental sessions over the course of 1 year. Sessions 1 and 2 involved baseline electrophysiological recordings and were held approximately 6 weeks apart. At sessions 3 and 4 blood samples were taken and questionnaires were administered. Session 3 was held midsemester, at a time free of exams. For most subjects, this session occurred between sessions 1 and 2. Session 4 occurred 24 h prior to each subject's self-reported most stressful final exam. Session 5 involved a series of blood draws taken before and after two affect eliciting film clips and took place approximately 6 months after session 1.

Electrophysiological Assessment, Sessions 1 and 2

Resting baseline EEG was collected from subjects at two separate experimental sessions, approximately 6 weeks apart. All subjects were run individually. The procedures followed closely those we described in previous studies (e.g., Tomarken et al., 1992b). After electrodes were applied for the recording of the EEG, computerized instructions informed subjects that: (1) there would be eight 1-min resting baselines; (2) four baselines would be conducted with eyes open, four with eyes closed; and (3) during the baselines, they should try to minimize eyeblinks and movements, but should not be so concerned about doing so that they were distracted.

Two randomly assigned counterbalanced orders were used for the eyes-open and eyes-closed trials of the resting baselines (O-C-C-O-C-O-O-C and C-O-O-C-O-C-C-O.) Subjects heard one tone denoting the start of each trial and two tones 60 s later to signal that the trial was over. After the first two baselines all subjects were reminded to stay relaxed and keep their eyes still. At this time, any subjects with excessive muscle tension or eye movements were provided relaxation techniques or tips to minimize eye movements. The remaining six baselines were presented consecutively without interruption.

Subjects returned to the laboratory for the second experimental session approximately 6 weeks after Session 1. The allowable interval between sessions was 39–45 days, with the vast majority of subjects returning 42 days after the first session. Most subjects were run at the same time of day for both sessions, but could be scheduled 2 h before or after their original appointment time. Procedures for the second session were identical to those for the first.

EEG Recording and Quantification

EEG was recorded according to procedures previously described (Tomarken et al., 1992b) using a lycra stretchable cap (manufactured by Electro-Cap Int. Inc., Eaton, OH) positioned on the subject's head using known anatomical landmarks. During each session, EEG was recorded from 29 electrode sites. All sites were recorded with a linked ears (A1,A2) reference. To remove those portions of the EEG that contained eye movement artifact, two channels of EOG were recorded: horizontal EOG (HEOG) was recorded from the external canthi of each eye; vertical EOG (VEOG)

was recorded from the supraorbit and suborbit of one (randomly selected) eye. All EEG electrode impedances were under 5000 ohm, and the impedances of homologous sites were within 1000 ohm of each other. EOG electrode impedances were under 20,000 ohm, with VEOG and HEOG impedances within 5000 ohm of each other.

EEG was amplified with a Grass Model 12 Neurodata System using Model 12A5 Preamplifiers, with bandpass filters set to 1 and 300, and no 60-Hz line filter in place. The signal was passed through MF6 digital anti-aliasing low-pass filters set at 200 Hz and characterized by a 36-dB/octave roll-off. EEG was digitized at 500 Hz by a 386DX2 25-MHz Dell PC and an Analog Devices RTI-815 A/D board.

The EEG and EOG signals were visually scored and those portions of the data containing eye movements, muscle movements, or other sources of artifact were deleted. When artifact occurred on a given channel, data from all channels were removed.

To calibrate the EEG, 25- and 50-μV 10-Hz sine waves were digitized on each channel. To assess the technical integrity of the recording system, additional calibration trials were run during which 4-, 20-, and 40-Hz 25- and 50-μV sine waves were digitized. These trials were run both immediately before and after each experimental session.

All artifact-free chunks that were 1.024 s in duration were extracted through a Hamming window, used to prevent spurious estimates of spectral power. Chunks were overlapped 50% to counteract the differential weighting of data points attributable to the use of the Hamming window. A fast Fourier transform (FFT) was then used to derive estimates of spectral power (in μV^2) in different frequency bands. The analyses presented in this article are restricted to the alpha band (8–13 Hz) since we have previously demonstrated that asymmetries in power in this frequency band account for the majority of variance in affect-related individual differences (Davidson, Chapman, Chapman, & Henriques, 1990) and our previous experiment that examined relations between NK activity and frontal asymmetry used power in the alpha band (Kang et al., 1991). Power density was computed by summing power values across each band and then dividing by band width.

In order to further aggregate the baseline data for analyses, a weighted mean of the eight baselines for each session was computed. When a subject had fewer than 10 s of artifact-free data for a given baseline, that baseline was dropped from further computations. All sessions had greater than 40 s of artifact-free data from each eyes-open and eyes-closed condition. Several steps were used in the computation of the weighted means. First, the power density across four eyes-open baselines was averaged, weighted by the number of 1.024-s chunks of artifact-free data. The same was done for eyes closed. Next, a simple average was computed across the two eye conditions. All power density values were log transformed in order to normalize the data. Finally, the simple average of power density values for sessions 1 and 2 was computed (see Tomarken et al., 1992b, for further details on this procedure).

When asymmetry data are reported, asymmetry is computed as the difference between mean log power density in a right hemisphere lead and mean log power density in a left hemisphere lead (i.e., log R minus log L power density).

Immune Sample, Session 3

This session was designed to provide a baseline immune sample. This session was held approximately 4 weeks following session 1. At a point during the semester that

was free of exams, subjects returned to the lab for a single blood draw. A trained phlebotomist collected approximately 15 ml of blood. Following this, subjects were asked to report the time and date of the final exam that they were most worried about and to answer a brief set of questions regarding whether or not they had smoked cigarettes, drunk alcohol, or used any other drugs the night before or the morning of their blood sample.

Immune Sample, Session 4

This session occurred approximately 6 weeks after the initial blood draw. Subjects were asked to return for their second immune session 24 h prior to their self-reported most stressful final exam. The mean interval between the blood draw and the final exam was 26.6 h. At this time, the phlebotomist again drew approximately 15 ml of blood. Following the blood draw, subjects filled out several questionnaires.

Film Immune Assessment, Session 5

Approximately 6 months after the initial baseline EEG session, 21 subjects agreed to return to the lab. At this session, subjects were shown two 30-min film clips designed to elicit positive and negative emotions. A phlebotomist drew a 15-ml blood sample before and after each film clip. The two film clips were presented in random order. The happy film clip was a medley of segments from ''Lady and the Tramp,'' the Winter Olympics, and ''Parenthood.'' The sad film clip, from the movie ''Beaches,'' depicted a mother dying of cardiomyopathy and her interactions with a childhood friend. Subjects rated their emotional responses to the films on a scale of 0 (none at all) to 9 (overwhelming) for the following emotions: Interest, Amusement, Happiness, Sadness, Fear, Disgust, and Anger. Each 30-min film was preceded by a brief synopsis on the video screen for the subjects to read. For the positive film clip, a synopsis was presented prior to each of the three segments. For the ''Lady and the Tramp'' segment (duration, 6.37 min), the synopsis was ''Lady and the Tramp enjoy a romantic spaghetti dinner under the stars after Lady gets her first peek at the baby.'' For the Olympics segment (duration, 16.05 min), the synopsis read ''Some moments from the U.S.A. at the Olympics.'' For the ''Parenthood'' segment (duration, 6.34 min), the synopsis read ''When the star attraction fails to show up at an anxious boy's birthday party, his father saves the day.'' For the sad film clip (duration, 29.12 min) the synopsis was ''A dying woman spends her last days with a childhood friend.''

These film clips were selected on the basis of ratings made by a minimum of 75 undergraduates during a series of film screenings. None of the subjects who screened film clips participated in the actual film study. Table I presents the data for the normative film ratings.

Blood Collection, Cell Preparation, and Natural Killer Cell Assay

Participants were asked to refrain from drinking caffeinated beverages on the morning of each blood draw. Fifteen milliliters of heparinized blood was collected for the NK assays. Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation over Histopaque 1077 (Sigma, St. Louis, MO). PBMC were washed twice in calcium- and magnesium-free Dulbecco's phosphate-buffered saline (DPBS) and resuspended in Iscove's modified Dulbecco's medium (IMDM) supplemented

TABLE 1
Ratings Used to Select Film Clips

Emotions	Lady/Tramp (n = 75)	Olympics (n = 144)	Parenthood (n = 134)	Happy medley (n = 144)	Beaches (n = 118)
Disgust	0.12	0.19	0.13	0.25	1.53
	(0.72)	(0.88)	(0.52)	(0.79)	(1.96)
Fear	0.05	1.25	0.14	0.54	2.99
	(0.23)	(1.89)	(0.51)	(0.97)	(2.30)
Sadness	0.72	1.13	0.45	1.29	6.51
	(1.17)	(1.68)	(0.82)	(1.44)	(2.12)
Anger	0.03	0.14	0.11	0.23	2.28
	(0.16)	(0.81)	(0.53)	(0.62)	(2.21)
Amusement	5.57	4.55	7.00	6.08	1.69
	(2.23)	(2.24)	(1.17)	(1.37)	(1.59)
Happiness	6.35	7.00	6.58	6.63	1.84
	(1.88)	(1.74)	(1.51)	(1.51)	(1.60)

Note. Rating scales ranged from 0 (no emotion) to 9 (overwhelming emotion). Standard deviations appear in parenthesis.

with 5% heat-inactivated fetal bovine serum, 1% GMS-S and 1% antibiotic/antimycotic solution (IMDM-C) (all from Gibco BRL, Grand Island, NY).

A standard chromium-release assay against K562 target cells was used. Briefly, 2×10^6 K562 cells were incubated with 100 μCi sodium chromate (^{15}Cr) at 37°C for 2 h, washed twice with IMDM-C, and resuspended at a final concentration of 2×10^4 cells/ml in IMDM-C. One hundred microliters of target cells and 100 μl of effector cells were added in quadruplicate to a 96-well round-bottom microtier plate at three effector:target cell ratios (11, 33, and 100:1). Spontaneous and maximal release were determined by incubating 100 μl of target cells with 100 μl IMDM-C or 2% cetrimide, respectively. After 4 h of incubation, supernatants were collected using a harvesting system (Skatron, Sterling, VA), and the released ^{15}Cr was quantified using a gamma counter (Packard-Cobra Series, Meriden, CT). Lysis was calculated using the formula % lysis = (experimental cpm − spontaneous cpm/maximal cpm − spontaneous cpm) × 100.

RESULTS

Baseline EEG Measures

Table 2 presents the descriptive EEG data derived from the baseline recordings averaged across sessions 1 and 2 that were used to examine relations between asymmetric anterior activation asymmetry and NK activity. The means were very similar to those of our previously published data on a large normative sample (Tomarken et al., 1992b).

NK Activity at Baseline and in Response to the Final Exam

Figure 1 presents the NK data at baseline and in response to the final exam for effector:target cell ratios of 33:1 and 100:1. At these two concentrations, NK activity was significantly lower during the final exam period than the baseline period (for

TABLE 2
Mean Alpha Band (8–13 Hz) Log Density and Asymmetry in Anterior Sites

	Region								
	Mid frontal			Lateral frontal			Anterior temporal		
	F3 (LPD)	F4 (LPD)	F4–F3 (ASYM)	F8 (LPD)	F8 (LPD)	F8–F7 (ASYM)	T3 (LPD)	T4 (LPD)	T4–T3 (ASYM)
M	1.28	1.31	0.03	0.60	0.64	0.04	0.63	0.71	0.08
SD	0.79	0.78	0.08	0.79	0.77	0.13	0.79	0.77	0.23

Note. LPD, linked-ears referenced log-transformed power density in μV^2/Hz (natural log). ASYM, asymmetry, calculated as log right minus log left power density. Power density values are means averaged across the two electroencephalographic sessions. Higher asymmetry scores indicate greater relative left hemisphere activation.

33:1, $t(23) = 2.52$, $P < .02$; for 100:1, $t(23) = 2.72$, $P < .02$). A similar trend was observed for the 11:1 ratio, though the difference was not significant ($t(23) = 1.09$).

Relations between Baseline Anterior Asymmetry and Baseline NK Function

To ascertain whether we replicated our earlier finding of relations between baseline measures of prefrontal activation asymmetry and baseline measures of NK activity, correlations between individual differences in anterior activation asymmetry measures and NK baseline measures (from the midpoint in the semester) were computed. In our previous study (Kang et al., 1991) we reported correlations between asymmet-

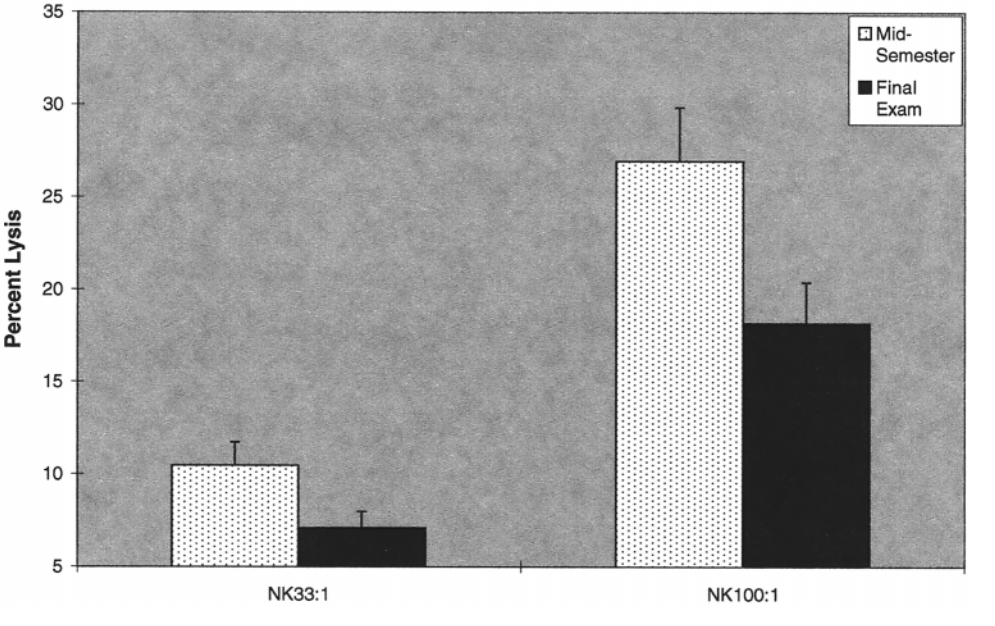

FIG. 1. NK activity (in percentage of lysis) at 33:1 and 100:1 effector:target cell ratios at the midsemester assessment (midsemester) and 24 prior to subjects' most important final examination (final exam).

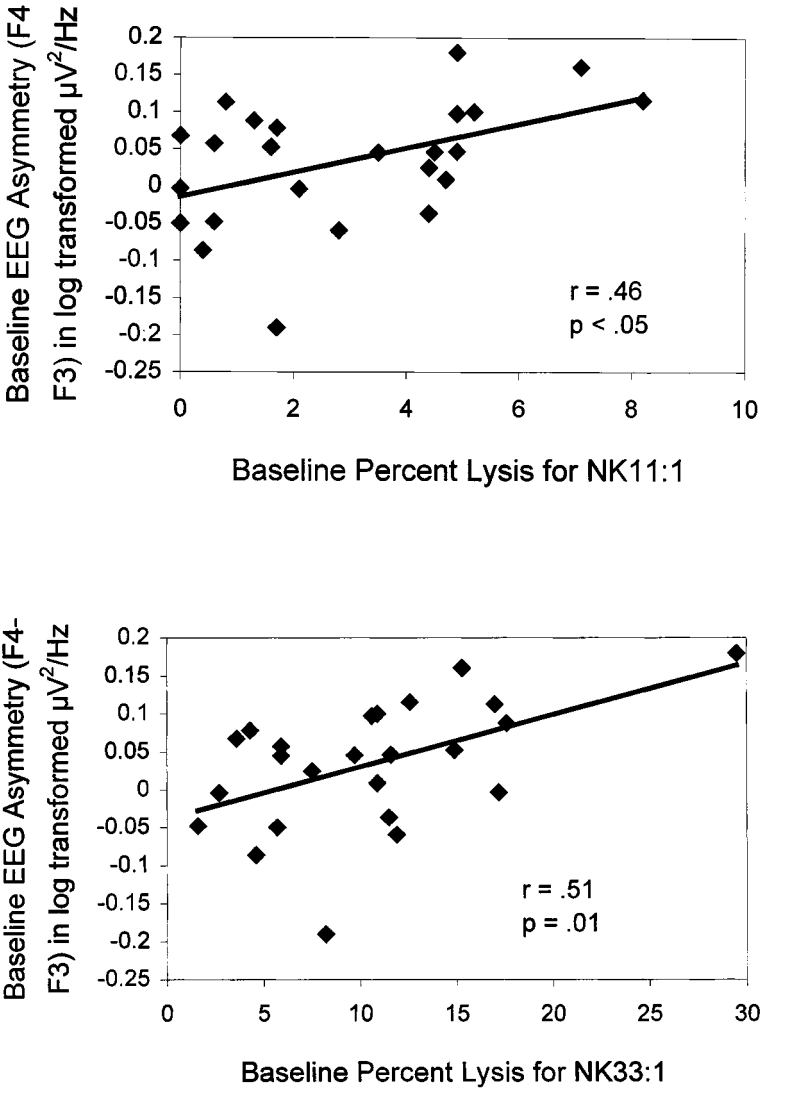

FIG. 2. Relation between baseline asymmetric prefrontal activation and NK activity at 11:1 and 33:1 effector:target cell ratios. The ordinate displays the asymmetry metric (right minus left log alpha power), where positive numbers denote relative left-sided activation and negative numbers denote relative right-sided activation. The abcissa displays the percentage of lysis.

ric activation in the mid frontal region derived from the F3/4 electrode pair. Correlations between prefrontal activation asymmetry derived from F3/4 and NK activity at 11:1 and 33:1 effector:target cell ratios were significant ($r = .46$ and .51, respectively, P's $< .03$; see Fig. 2), indicating that subjects with greater relative left-sided prefrontal activation had higher levels of NK activity. The correlation at the 100:1 ratio was in the same direction but not significant ($r = .37$, $P = .07$).

Activation asymmetries at both lateral frontal (F7/8) and anterior temporal (T3/4) regions were also related to NK activity in the same manner as that observed for

the mid frontal region. At the 11:1 effector:target cell ratio, both lateral frontal and anterior temporal asymmetry were significantly correlated with NK activity ($r = .41$ and .48, respectively, P's $< .05$). The correlations for the other effector:target ratios were all in the same direction but none were significant.

To determine whether the relations we observed between asymmetric activation and NK function were specific to the anterior regions, we also computed correlations between NK activity and asymmetric activation derived from all other homologous sites. The correlations in the posterior scalp regions were low and nonsignificant. For example, the average correlation between asymmetric activation in the parieto-occipital region (PO3/4) and NK activity (averaged across the three effector:target ratios was .13. Figure 3 presents a topographic map of the correlations between asymmetric activation across the entire scalp and NK activity at the 33:1 effector:target ratio. From this figure, it can be seen that relation between activation asymmetry and NK function is specific to the anterior scalp region.

Predicting NK Reactivity to Final Exam Stress from Baseline Anterior Asymmetry

Baseline measures of anterior activation asymmetry were unrelated to measures of NK function at the time of the final exam. To determine whether baseline measures of anterior activation asymmetry predicted the change in NK function from mid-semester to the final exam period, a series of hierarchical regressions were computed for the mid frontal and lateral frontal asymmetry measures. The dependent variable in these analyses was the final exam NK measure. The first step in the regression equation was the NK measure at midsemester (baseline NK). The second step was the asymmetry measure. We found that at the 100:1 effector:target ratio, individual differences in lateral prefrontal activation asymmetry accounted for significance variance in the final exam NK measure, following the statistical removal of baseline NK activity. Baseline NK activity accounted for 6.0% of the variance in final exam NK activity. The lateral frontal asymmetry measure, entered as step two in the regression model, accounted for an additional 21.1% of the variance [$F(2,23) = 6.06$, $P = .02$]. The direction of this relation indicated that subjects with more relative left-sided activation at baseline showed a smaller decline in NK activity in response to the final exam stress. The regression for the mid frontal scalp region revealed a similar pattern but was not significant.

In order to ascertain whether the variance in final exam NK activity accounted for by prefrontal activation asymmetry was because of the association between anxiety and prefrontal activation asymmetry, we computed two additional hierarchical regressions where we entered either the change in state anxiety from baseline to the final exam period or the change in trait anxiety between these periods as the first predictor and the asymmetry measure as the second predictor. In each case, the anxiety variable

FIG. 3. Spline-interpolated topographic map of the relation between NK activity (at 33:1 effector:target ratio) and asymmetric activation across the scalp. Color scale depicts actual *r* values of the correlations at different scalp regions. The red end of the spectrum depicts the highest correlations and the blue end the lowest correlations. As can be seen from this figure, the strongest relations are present for the frontal and anterior temporal regions. Little relation between asymmetric activation and NK activity is present in the posterior scalp regions.

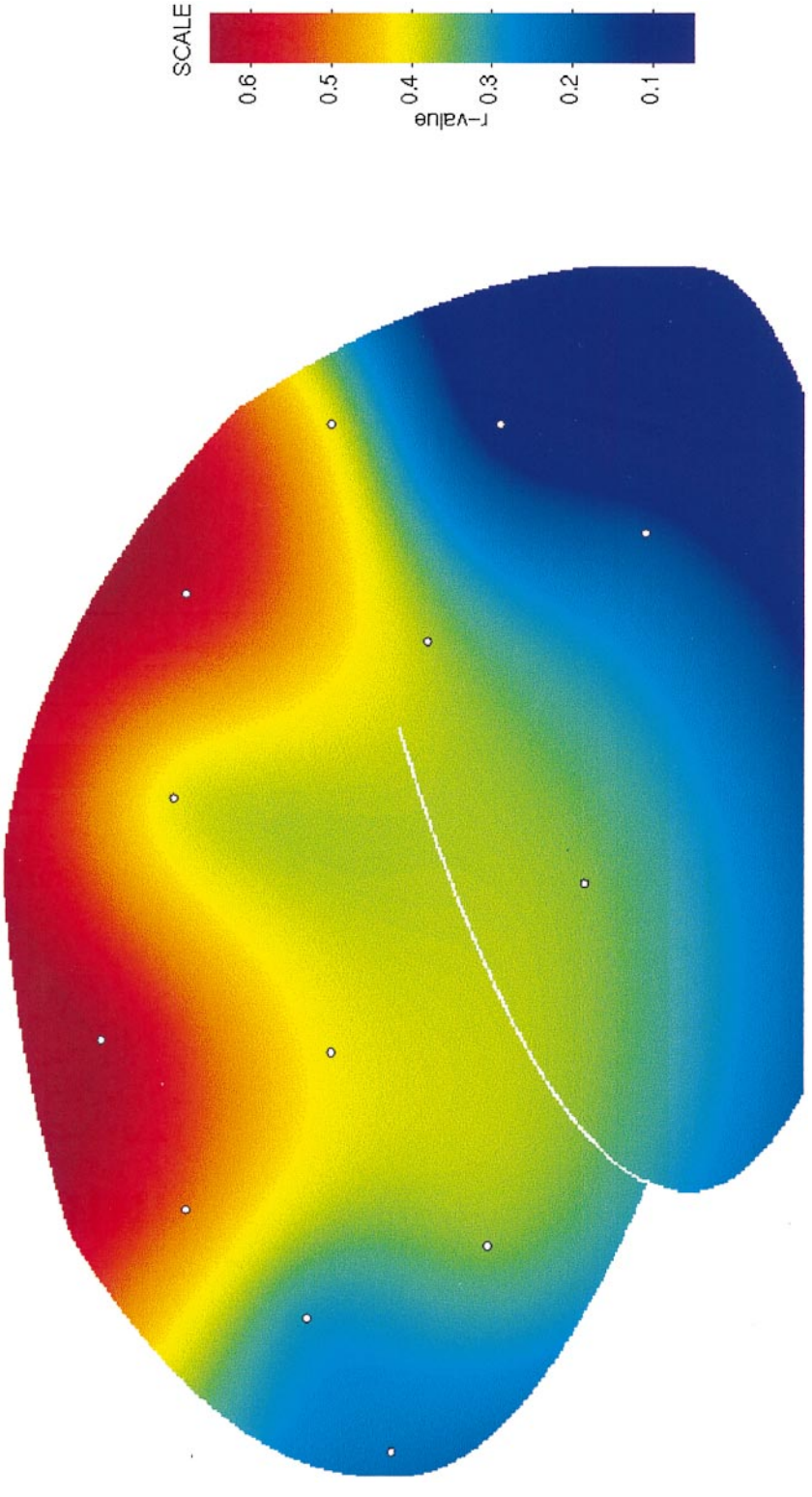
SCALE
0.6
0.5
0.4
0.3
0.2
0.1
r-value

TABLE 3
Hierarchical Regressions Predicting Post-Positive Film NK Function

	% Unique variance	*F* value	*P* value
11:1			
Step 1: NK function prefilm	75.5	58.65	<.0001
Step 2: Lateral prefrontal asymmetry	6.2	6.07	.02
33:1			
Step 1: NK function prefilm	73.2	56.77	<.0001
Step 2: Lateral prefrontal asymmetry	5.2	4.27	.05
100:1			
Step 1: NK function prefilm	81.5	83.60	<.0001
Step 2: Lateral prefrontal asymmetry	3.3	3.90	.05

accounted for less than 1% of the variance and the asymmetry variable remained significant following the removal of variance associated with anxiety.

Effect of Positive and Negative Films on NK Function

The effect of the films on emotion ratings were first examined to establish that the clips did indeed arouse the intended positive and negative emotion. There was no difference between the positive and negative clip on interest ratings (mean, 6.0 for both). Subjects reported significantly more happiness ($t(20) = 13.93$, $P < .0001$) and amusement ($t(20) = 5.15$, $P < .0001$) in response to the positive clip than to the negative clip. In response to the negative clip subjects reported significantly more sadness ($t(20) = 16.31$, $P < .0001$), fear ($t(20) = 4.34$, $P < .0005$), and anger ($t(20) = 3.87$, $P < .001$) than in response to the positive clip.

To determine whether the films produced any group-level change in NK function, we tested whether NK function determined from the blood draw immediately after the positive and negative films differed significantly from the NK baseline measure taken at the start of the film session. There were no significant differences pre- to postfilm for any of the NK measures (all t's < 1).

Relations between Baseline Anterior Asymmetry and NK Reactivity to Emotional Film Clips

To assess the relation between baseline EEG collected at sessions 1 and 2 and the natural killer cell activity occurring during the film session, a series of hierarchical regressions were computed that were structured identically to those reported above for the prediction of final exam NK function. For the emotional films, we computed separate regressions to predict NK function post-positive and post-negative film. The first step in the equation was always the corresponding NK measure obtained at the start of the film session (baseline NK for the film session). The second step was the asymmetry metric. These regressions were computed for the mid frontal and lateral frontal electrode sites. In response to the happy film, subjects with greater relative left-sided prefrontal activation showed more of an increase or less of a decrease in NK function pre- to postfilm. This effect was significant for the lateral frontal region at each of the effector:target ratios (see Table 3). It was in the same direction for each effector:target ratio for the mid frontal region but was significant

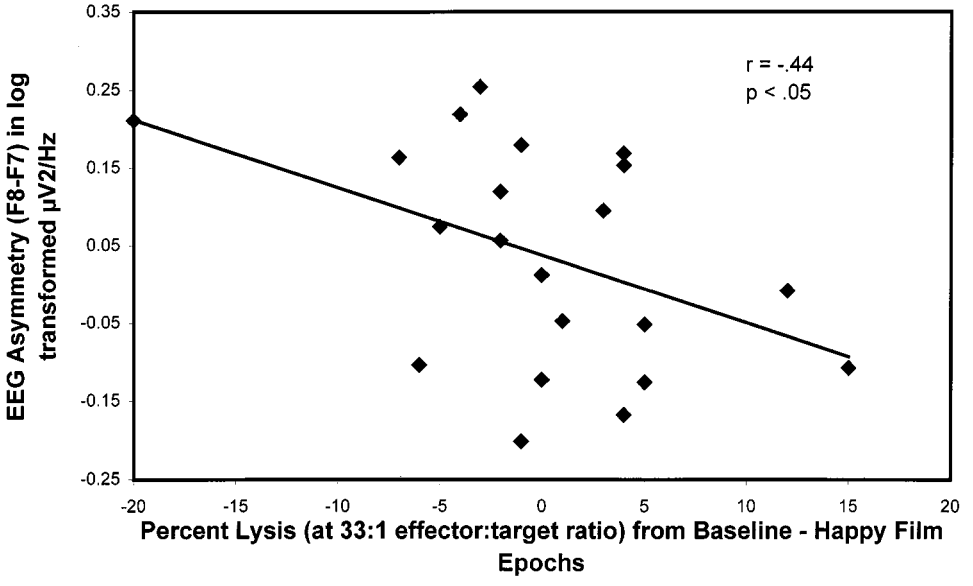

FIG. 4. Relation between baseline asymmetric lateral prefrontal activation and the change in NK activity (33:1 effector:target ratio) from baseline to just after the positive film clip. Positive numbers on the abscissa reflect lower NK activity during the happy film compared with baseline, while negative numbers indicate higher NK during the film compared with baseline. The ordinate displays the asymmetry metric where higher numbers denote greater relative left-sided activation.

only for the 100:1 ratio. The general pattern these regressions identify is illustrated in Fig. 4, where the correlation between lateral prefrontal activation asymmetry and the change score from baseline to post-positive film is depicted in a scatter plot. As can be seen from this figure, subjects with greater relative left-sided activation show an increase in NK function from pre- to postfilm ($r = -.44$, $P < .05$).

In order to examine whether the lateral frontal asymmetry variable still accounted for a significant portion of variance in NK change in response to the positive film clip after removing the variance in NK change accounted for by self-reported changes in positive and negative affect, additional hierarchical regressions were computed where the first step in the model was NK activity at baseline, the second step was ratings of positive and negative affect following the film clip, and the third step was the asymmetry variable. These regressions revealed that the lateral frontal asymmetry variable still accounted for a significant or near-significant portion of variance in the postfilm NK measure after removing the variance accounted for by self-reported changes in positive and negative affect (for 11:1, $p = .03$; for 33:1, $p = .06$; for 100:1, $p = .03$).

The identical regressions were computed for the prediction of NK activity following the sad film. While the direction of these effects consistently indicated that subjects with greater right-sided prefrontal activation at baseline had more of a decline in NK function following the sad film, none of the effects reached significance.

DISCUSSION

We hypothesized that individual differences in baseline measures of prefrontal activation asymmetry would predict basal differences in NK function, based upon

our earlier findings (Kang et al., 1991). This hypothesis was confirmed. Subjects with greater relative left-sided anterior activation (in mid frontal, lateral frontal, and anterior temporal regions) showed higher levels of NK activity. Individual differences in asymmetric activation from posterior scalp sites were unrelated to differences in NK function, underscoring the specificity of this effect to anterior brain regions.

We also hypothesized that individual differences in baseline measures of prefrontal activation asymmetry would predict the magnitude of change in NK function following both naturally occurring (final exam) and experimentally induced (positive and negative emotional film clips) emotional challenge. Our findings indicate that measures of prefrontal activation account for a substantial portion of variance in the decline of NK function during the final exam period. Lateral prefrontal activation asymmetry accounted for 21% of the variance in NK function at final exam time, after removing the variance predicted by baseline NK function such that subjects with greater relative right-sided activation had lower levels of NK function at this time. In response to the less emotionally challenging film clips, we found that subjects with greater relative left-sided lateral prefrontal activation exhibited significantly higher levels of NK activity following the positive film after removing the variance in postfilm measures of NK activity predicted by baseline NK activity. This effect, however, accounted for only a maximum of 6.2% of the variance, in contrast to the final exam case, where we were able to account for more than three times the proportion of variance with the measure of prefrontal activation asymmetry. Importantly, these effects remained after removing the variance in NK activity accounted for by changes in self-reported emotion in response to both the final exam stress and the film clips. While this pattern of findings indicates that the electrophysiological measures of anterior activation asymmetry account for variance in NK activity beyond that accounted for by self-reported changes in emotion, it should be emphasized that these data do not necessarily imply that the relation between asymmetric anterior activation and NK function is independent of emotion. In other work we have demonstrated that electrophysiological measures of prefrontal activation asymmetry are sensitive to parameters of emotional reactivity and regulation that are not necessarily represented in self-report (see Davidson, 1998b). Thus, the individual differences in anterior activation asymmetry that are featured in this study may index certain features of emotional responding that are not well represented in the self-report measures that were obtained.

While many of the effects we described were present at all effector:target cell ratios, some were significant only for one or two ratios but not all three. For the relations between asymmetry and basal NK activity, as well as for reactivity to the positive films, the associations we observed were significant for at least two of the effector:target ratios. It should be noted, however, that the pattern of association was always the same across the three ratios tested.

Together, these findings support and extend existing literature on the construct validity of measures of prefrontal activation asymmetry. They replicate our earlier findings on the association between individual differences in prefrontal activation asymmetry and basal NK function (Kang et al., 1991). The current study extends these earlier findings by demonstrating that individual differences in anterior asymmetry also predict NK reactivity to both naturally occurring and experimentally induced emotional challenges. Subjects with greater relative right-sided prefrontal activation show larger magnitude reductions in NK function during the stress of final

examinations and they show less of a rise in NK function in response to a positive film clip.

The mechanisms accounting for the association between asymmetric prefrontal activation and NK function remain unclear. However, recent evidence of an association between asymmetric prefrontal activation and cortisol (Kalin et al., 1998) suggests that one mechanism may be cortisol-mediated (see Cacioppo, 1994). Further research is necessary to fully clarify the nature of this relation. The findings from this study underscore the fact that at least some of the variance among individuals in NK function is lawful and can be accounted for by individual differences in measures of prefrontal activation asymmetry.

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