ity to exclude trypan blue and to multiply subsequently in the presence of complete culture medium. A binding and release procedure taking a total of 2 hours increased the percentage of trypan blue–positive cells by about 5 percent over control values, whereas control cells kept under the same conditions but without LCL-beads showed an increase of only about 1 percent over the initial value. During the binding it was also observed that, in general, the percentage of trypan blue–positive cells in the unbound fraction increased. Released cells multiplied in complete medium 1.54-fold in 24 hours, whereas control cells increased 1.65-fold. The multiplication rate during the following 2 days was almost identical in both groups. Sterility was controlled by antibiotics.

In summary, the results demonstrate that immobilized LCL induces tissue culture cells to bind to a solid matrix. This is prevented by hapten sugars specific for LCL. The cell-bead bond seems to be strong enough to resist appreciable mechanical removal of cells. The binding occurs sufficiently fast that the release procedure can be commenced before considerable secondary interactions between cell and bead have taken place, as suggested by Edelman *et al.* (*3*). Unlike immobilized Con A and wheat germ agglutinin, LCL allows the sugar-specific release of cells within a period of time short enough to maintain the viability of nearly all cells. It remains to be evaluated whether immobilized LCL permits cell separations on the basis of cell surface differences, such as those carried out with other lectins.

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Facial Muscle Patterning to Affective Imagery in Depressed and Nondepressed Subjects

Abstract. *When subjects imagine happy, sad, and angry situations, different patterns of facial muscle activity are produced which can be measured by electromyography. These subtle, typically covert, facial expression patterns differentiate depressed from nondepressed subjects. Facial electromyography can provide a sensitive, objective index of normal and clinical mood states.*

It is a common human experience that thoughts or memories can elicit specific emotions or feeling states. The ability to experience various emotions depends in part on the person's mood and it is not unusual, for example, for a person who is depressed to describe having difficulty imagining happy situations and generating a positive feeling state. In the past researchers have relied almost exclusively on self-report as the means of assessing a person's differing moods. We report here the results of an experiment with both nondepressed and depressed subjects illustrating a psychophysiological procedure for indexing subtle emotional states.

The face has long been associated in both lower animals and man with the concept of innate, fundamental emotions (*1*). Recent cross-cultural data (*2*, *3*) document that at least six distinct emotions can be recognized in the human face: happiness, sadness, anger, fear, surprise, and disgust. In light of the unique speed and sensitivity with which the facial musculature responds (*4*) we reasoned that it would be possible to record discrete patterns of low-level muscle activity during the generation of affective imagery, even though the facial adjustments might be too small or rapid to be visually detected by the average observer. Prior research using electromyographic procedures has shown that covert muscle changes often accompany cognitive processes that are associated with different motor movements [for example, when subjects engage in silent reading, small muscle changes can be recorded from the lip region (*5*)]. This phenomenon has not been systematically studied in relation to different emotions elicited by imagery.

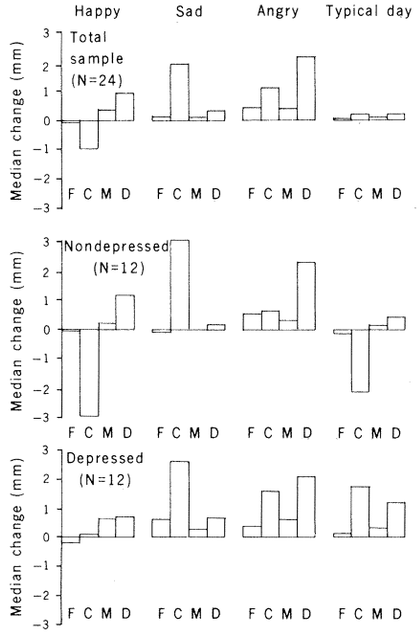

Fig. 1. Median EMG change scores from resting baselines for frontalis (*F*), corrugator (*C*), masseter (*M*), and depressor (*D*) regions during imagery conditions for the total sample and the nondepressed and depressed subsamples. One millimeter equals 45 μv per 30 seconds.

We recorded electromyographic (EMG) activity from selected regions of the face using miniature Beckman Ag-AgCl electrodes placed adjacent to each other in pairs with interelectrode resistance reduced to less than 3000 ohms. Surface electrodes have certain advantages over intramuscular fine wires in that they minimize stress to the subject, introduce little risk of infection, and can be readily placed over the same area for repeated measurement. The limitation of surface electrodes is that the recording is potentially affected by cross talk from adjacent muscles, making inferences about the activity of anatomically specific muscles subject to qualification (*4*). Although for convenience specific muscles are referred to in this report, the data actually reflect activity from muscle areas or regions. Since the method proves to be internally consistent in its

application and is differentially sensitive to affective imagery, its use as an empirical index of facial expression and emotions need not require that it directly correspond to the classical functions of the inferred underlying muscles.

The EMG activity is recorded by Grass wide-band a-c preamplifiers at 7.5 μv per millimeter, displayed on a four-channel Tektronix oscilloscope, and recorded on FM tape. It is rectified and integrated with Grass 7P10B integrators and displayed on a model 78B polygraph as a ramp function, with 1 mm of deflection calibrated to equal 45 μv. Subjects recline with eyes closed in a comfortable lounge chair in a sound- and temperature-controlled room. The subject's overt facial expression is monitored by one video camera; a second video camera views the oscilloscope. By means of a Panasonic special effects generator, the overt face and the EMG are displayed side by side on a video monitor and recorded on video tape. This makes it possible to directly compare overt behavior to the EMG activity and also to monitor for possible artifacts.

In the experiment, four pairs of electrodes were placed on the right side of the face (*6*), over the corrugator and frontalis regions of the eyebrow and forehead and the depressor anguli oris and masseter muscle regions of the mouth and jaw (*7*). Twenty-four female subjects between 19 and 58 years of age were studied; 12 volunteer subjects with scores on the Zung Self Rating Depression Scale (*8*) below 50 (classified as normal) (mean = 37), 6 volunteers with Zung scores above 50 (classified as depressed) (mean = 58), and 6 patients with Zung scores above 56 (mean = 75) who were about to begin drug therapy for depression. All subjects received a within subject design consisting of covert imagery trials followed by overt voluntary facial expression trials. After a 5-minute resting baseline, subjects were told to imagine happy, sad, and angry situations which had strongly evoked these specific emotions in their past. Each emotion was imagined for 3 minutes, with the instruction to attempt to reexperience the feelings associated with the imagery. Initially included as a non-emotional control trial, subjects were also asked for 3 minutes to simply "think about what you do in a typical day" (from awakening in the morning to going to sleep at night) with no requirement to re-experience any particular emotion. Order of imagery trials was counterbalanced across subjects, and 2-minute rest periods occurred between each imagery condition.

Following a final 3-minute post-imagery resting baseline, subjects were asked to make and hold, for 30-second periods, voluntary facial expressions depicting happiness, sadness, and anger. These data were used to verify that the electrodes differentiated between the four muscle regions and to determine what the specific EMG patterns were for each of the three overt expressions (*9*). At the end of the experiment, subjects filled out a short version of Izard's Differential Emotions Scale (DES) (*10*) to assess the extent to which they experienced success in generating the required emotions during the imagery conditions.

To minimize subject awareness of the purpose of the experiment, the 18 volunteer subjects were told that the purpose of the experiment was to measure general body movements during affective imagery and that the purpose of the TV camera was to observe whole body (rather than facial) movement. Additional pairs of Beckman electrodes were attached to (but did not actually record from) the subject's arms and legs, further implying that many muscle sites were being monitored physiologically. Since the voluntary facial expressions always followed the imagery trials, they could not influence imagery results.

Results for the four imagery conditions are displayed in Fig. 1 for the total sample and separately for the nondepressed and depressed subjects. On the basis of their Zung scores and the high degree of similarity of their patterns of EMG activity during imagery, data from the six depressed volunteers are combined with the data from the depressed patients to form a depressed subject group. The data are based on change scores from resting baseline of integrated EMG activity averaged over 30-second periods for the 3-minute trials. Since the scores did not always yield normal distributions, the data are plotted as group medians, and nonparametric statistics (*11*) are used to evaluate the data. Tables 1 and 2 provide values from Wilcoxon matched-pairs signed-ranks tests (two-tailed) for changes from baseline and differences across imagery conditions, for the total sample and each group separately. The P values reflecting the direct comparisons between the two groups are based on Mann-Whitney U tests (two-tailed).

The visual appearance of the curves is supported by statistical analyses. For the total sample, each discrete imagery period produces a different EMG pattern. Happiness is associated with decreased corrugator ($P < .05$), increased masseter ($P < .01$), and increased depressor

Table 1. P values (two-tailed) for the four imagery conditions and four muscle regions. Values are based on changes from baseline per muscle per imagery condition for the total sample ($N = 24$) and for the nondepressed ($N = 12$) and depressed ($N = 12$) subsamples separately; comparisons are also made between the two subsamples. Blank spaces reflect P values greater than .10 (see text). Abbreviations: F, frontalis; C, corrugator; M, masseter; and D, depressor.

Sample	Happy				Sad				Angry				Typical day			
	F	C	M	D	F	C	M	D	F	C	M	D	F	C	M	D
Total		.05	.01	.01		.06		.02	.01	.05	.01	.01				.05
Nondepressed		.02	.05	.01					.05		.05	.01	.02			
Depressed			.07	.05	.05	.05		.07	.09	.05		.01		.07		.02
Nondepressed versus depressed		.09			.05								.06	.08		

Table 2. P values (two-tailed) for the four imagery conditions and four muscle regions: comparisons between pairs of imagery conditions within muscles for the total sample and for the two subsamples separately. Blank spaces reflect P values greater than .10 (see text). Abbreviations: H, happy; S, sad; A, angry; and TD, typical day.

Sample	Frontalis			Corrugator			Masseter			Depressor		
	H	S	A	H	S	A	H	S	A	H	S	A
Total												
S	.05			.01			.02					
A	.01			.01				.06			.05	
TD			.05	.01	.02	.05	.01		.02			.01
Nondepressed												
S				.01								
A	.05			.01							.02	
TD			.02	.02	.02	.07	.05		.02	.07		.01
Depressed												
S	.01			.05								
A				.10								
TD	.06			.05								

($P < .01$). Sadness is associated with increased corrugator ($P < .01$) and increased depressor ($P < .02$), and anger is associated with significant increases in all four placements, particularly over the depressor region. Note that "typical day" for the group as a whole is associated only with increased depressor activity ($P < .05$), and the magnitude of change is very small. Comparisons across the three emotions within each muscle further clarify these patterns, showing that the corrugator and frontalis each differentiate happiness from both sadness ($P < .01$ and $< .05$) and anger ($P < .01$ and $< .01$), whereas the masseter and depressor each differentiate sadness from anger ($P < .06$ and $< .05$).

Analyses of the separate groups provides further support for the idea that facial EMG activity is sensitive to mood. The curves suggest that whereas both groups show similar sadness and anger patterns, the depressed subjects show an attenuated pattern for happiness. Furthermore, the typical day EMG pattern for the nondepressed subjects looks like a miniature happy pattern, while for the depressed subjects it is more similar to a sad pattern. Examination of the change scores from baseline indicates that the nondepressed subjects more reliably generate a happy pattern than the depressed subjects (for corrugator $P < .02$ for nondepressed, nonsignificant for depressed); the reverse is the case for the sad pattern (that is, for frontalis $P < .05$ for depressed, nonsignificant for nondepressed). Direct comparisons between the groups support this general conclusion: the nondepressed subjects tend to produce a greater decrease in corrugator during happy imagery ($P < .09$), the depressed subjects produce a greater increase in frontalis during sadness ($P < .05$), and these effects tend to be combined and repeated in the typical day condition ($P < .06$ and $P < .08$, respectively). It is important to note that of the 24 EMG comparisons, the nondepressed subjects exhibit 12 that are significant at least at $P < .10$, while the depressed subjects produce only 5 that are significant at this level (this difference between groups is significant on the Fisher exact test at $P < .01$). The findings that nondepressed subjects (i) specifically produce a more consistent happy imagery EMG pattern than depressed subjects and (ii) produce a significantly ($P < .01$) greater number of $P < .10$ changes from baseline in EMG during imagery conditions in general than depressed subjects have been replicated and extended in a subsequent experiment (*12*).

Analysis of the DES indicates that the subjects experienced success in generating affect-specific images. Importantly, the subjective reports between the two groups generally mirror the differences uncovered in the facial EMG; on a scale from 1 to 5 the nondepressed subjects report more happiness during happy imagery (4.2 compared to 2.6, $P < .002$) and typical day (3.0 compared to 2.5) and less sadness during typical day (1.2 compared to 2.8, $P < .002$).

Examination of the video tapes reveals that the facial EMG patterns found during imagery are not readily detected on the overt face. This is not surprising since correct classification of more gross facial changes, especially those occurring quickly over time, is often difficult and unreliable (*2*). However, subjects do differ in the degree to which their faces show lability during imagery, and it is conceivable that a person specially trained to visually score selected muscle regions and patterns of regions could approximate the major EMG conclusions. It remains to be determined whether facial EMG provides a more reliable or relevant index of emotion under selected experimental conditions than self-report (*10*) or visual observation (*13*) techniques.

These data provide new psychophysiological support for the theory of differential emotions (*1–3, 14*), including affect elicited by imagery. This theory postulates further that peripheral feedback from discrete, innate patterns of facial muscle activity provides an important component underlying the subjective experience of emotion. Applying this to imagery, we hypothesize that a self-regulated internal feedback loop may be created, with the particular image triggering a specific pattern of peripheral changes which, in turn, is reprocessed by the brain, contributing to the unique, emergent feeling state associated with the imagery (*15*). This hypothesis is indirectly supported by the findings for depressed subjects, since the attenuation in their ability to self-generate a positive feeling state is mirrored in their facial EMG (*16*). Taken together, the data suggest that facial EMG can provide a sensitive and objective procedure for indexing normal and clinical mood states.

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7. These placements were selected to potentially top sadness (corrugator, frontalis, and depressor) and anger (corrugator and masseter) as inferred from overt facial expressions with eyes open (*1–3*). However, since the subjects in this experiment had their eyes closed and were in a reclining posture, and surface electrodes were used to record EMG activity, it is not appropriate to directly compare these EMG patterns with those derived from the prior facial expression literature.
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9. Depressed and nondepressed subjects generated comparable EMG patterns during voluntary overt facial expressions. These data, coupled with the pattern of results obtained during the imagery conditions, suggest that the differences found during imagery between the depressed and nondepressed subjects do not reflect simple differences in motivation to perform due to experimental demand characteristics. It should be further noted that the issue of situational demand characteristics could only apply to half the depressed sample (the six patients); the volunteer subjects all entered the experiment as "normal" subjects and did not know that their status might later be changed to symptomatic depressed based on the outcome of post-experimental questionnaires.
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12. In the second study an additional muscle placement—the zygomatic major—also differentiated between nondepressed and depressed subjects during "think happy" conditions ($P < .02$). Since the zygomatic is intimately involved in the generation of a smile (*2*), the finding for happy imagery may be consistent with the underlying anatomy (G. E. Schwartz, P. L. Fair, P. Salt, M. R. Mandel, G. L. Klerman, *Psychosom. Med.*, in press). In a third experiment, it has been found that depressed patients who evidence changes in corrugator activity after 2 weeks of antidepressant (or placebo) treatment, show corresponding changes ($P < .01$) in clinical improvement (G. E. Schwartz, M. R. Mandel, P. L. Fair, P. Salt, M. Mieske, G. L. Klerman, in preparation).
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