Respiratory Sinus Arrhythmia in Persons with Organic Solvent Exposure:
Comparisons with Anxiety Patients and Controls

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ABSTRACT

Persons with exposure to organic solvents have been shown to have psychiatric symptomatology as well as cognitive impairments. Several studies have suggested that some of the psychiatric symptoms in these patients reflect similar reactions observed in patients with anxiety disorders. Respiratory sinus arrhythmia (RSA) provides an indication of impaired autonomic functioning. Whereas decreased RSA has been reported in anxiety patients, it has also been noted that higher respiratory rates in these patients obscures differentiation of RSA from normal values. We recorded multiple parameters of RSA under a paced-breathing condition in 28 patients with solvent exposure, 18 patients having anxiety disorder, and 31 controls. High one-year retest reliability was observed for a subset of the subjects. Significantly reduced RSA was observed for both anxiety and solvent-exposed patients as compared to controls. Maximum mean heart rates/cycle did not differ among groups, but minimum heart rates were significantly lower for controls than for the two patient groups. The findings suggest that the reduced RSA among patients is not related to higher maximum rates, but rather to a decrease in vagally-mediated alteration associated with respiratory changes that is observed in both types of patient groups.

RUNNING TITLE: RSA in Solvent Exposure and Anxiety

KEYWORDS: Respiratory sinus arrhythmia, solvent exposure, anxiety, heart rate variability
INTRODUCTION

Individuals with toxic exposures to organic solvents exhibit both cognitive deficits and emotional lability (1,2). It has been suggested that some of the personality variation in these patients reflects similar reactions observed in patients with anxiety disorders, including posttraumatic stress syndrome (3). Alterations in emotional expressivity are strongly associated with the autonomic nervous system (ANS). Previously, cardiac and pupillary measures obtained during cognitive tasks indicated impairment of ANS functioning in persons with a history of exposure to organic solvents (4). These findings suggest that further measures of ANS function also could indicate impairment in such individuals.

Respiratory sinus arrhythmia (RSA) is a well studied measure of ANS activity (5). RSA refers to changes in heart rate produced by inhalation and exhalation during the respiratory cycle. Various terms and measures are used to represent RSA, such as differences in maximum and minimum rates during respiratory cycles, absolute change between successive beats, and estimates of variance based on these measures, although these measures are highly intercorrelated (6). Assessment of RSA depends upon accurate determination of time between successive heart beats, obtained from the time of the R-wave of the electrocardiogram (ECG). The time between beats is termed interbeat interval (IBI) or R-R interval.

Changes in heart rate linked to respiratory activity are produced by activation of the parasympathetically-mediated vagal nerve. Greater vagal activation leads to greater increases in interbeat interval, which is therefore reflected as decreased heart rate (5). Thus, decreased RSA represents decreased modulation of vagal activity, and provides a clinical indication of impaired parasympathetic function. Murata and colleagues have conducted several studies of workers exposed to such solvents as xylene, toluene, n-hexane, and styrene, reporting reductions in measures of RSA among these workers (7-10).

Changes in RSA are often elicited in the clinical setting by having the subject perform the Valsalva maneuver, which elicits high respiratory volume through deep breathing (11). Paced breathing has previously been employed to study RSA in a number of experimental
paradigms (12-14). Respiration is often assessed during such procedures. However, recording of additional psychophysiological measures (not reported here) precluded direct assessment of respiration. One of the objectives of the present study was to examine the use of normal levels of respiration when pacing was indicated to the subject by an external cue.

Patients with anxiety disorders exhibit decreased RSA (15, 16). Anxiety is also a clinical feature presented by persons with organic exposure (17). The primary objective of the current study was to determine the extent to which similar alterations of RSA were evidenced in both types of disorders. A finding of similar patterns of impairment in both groups would suggest a communality contributing to the final autonomic pathway related to these measures.

METHOD

All subjects recruited for this study participated in a comprehensive series of neuropsychological, psychophysiological, and psychiatric diagnostic evaluations, which were almost always conducted on a single day. In addition, all subjects completed the SCL-90-R, a self report inventory assessing symptoms of psychopathology, including anxiety (18). Assessment included a comprehensive history of chemical exposure in the workplace and home, and psychiatric evaluation of DSM-IV Axis I and Axis II symptomatology utilizing well established semi-structured interviews (SCID and PDE). This paper examines the cardiac modulation associated with respiratory cycles cued by an external stimulus.

Individuals with a history of exposure to organic solvents meeting criteria for Type 2A/2B solvent encephalopathy (cognitive and mood changes) were referred for participation in the study after evaluation by the Dept. of Occupational and Environmental Medicine, University of Pittsburgh School of Medicine. Twenty-eight consecutive referrals (mean age = 44.3 years, s.d. = 9.9) were evaluated. Non-exposed controls (n = 31, mean age = 40.0, s.d. = 11.3) were recruited as a primary comparison group. These individuals had no history of chemical exposure and were free of psychiatric disorder or medical complications such as diabetes, or neurological
disorder (mean age = 44.3 years, s.d. = 9.9). A second comparison group consisted of individuals who were similar to the non-exposed group but who met DSM-IV criteria for General Anxiety Disorder (n = 18, mean age = 41.0, s.d. = 9.2) and were referred by clinicians of the Department of Psychiatry. All diagnoses were independently confirmed based on the research interviews and case conferences conducted by our research group. The groups did not differ significantly with respect to age. All subjects were evaluated only after informed consent was obtained; subjects were paid for their participation in the protocol. The study was approved by the IRBs of the University of Pittsburgh School of Medicine and the VA Pittsburgh Healthcare System.

Within the exposed group, the average exposure duration was 6.3 years (range 1 day to 30 years). All subjects had been exposed to mixtures of organic solvents (e.g., toluene, benzene, xylene) and the primary route of exposure was inhalation, although some had direct dermal contact with the solvents as well. The average exposure-to-test interval was 18 months (range 1 week to 84 months). One-third of the subjects reported at least one peak exposure -- an episode in which they had been exposed to an excessive amount of solvent that resulted in a visit to a doctor, or hospitalization. Given the wide range of exposure histories, subjects were ranked on a scale of 1 to 3. Those rated as 1 had the mildest exposure (i.e., shortest duration of exposure, no history of peak exposure with acute treatment). Those rated 3 had longest durations of exposure and/or a history of peak exposure with treatment. Persons who fell between these limits were rated 2.

Prescribed medications being taken by the patients were grouped as antidepressants and anxiolytics (12 solvent and 12 anxiety patients), blood pressure medications (5 solvent patients), or no medications other than antacids (11 solvent and 6 anxiety patients).

As part of the psychophysiological battery, respiratory sinus arrhythmia was evaluated from the electrocardiogram (ECG). Subjects sat in a dimly illuminated room, separated from the experimenter's control room, and looked at a small red light-emitting diode (LED). They were
told that the LED would be turned on for three sec, and then off for three sec, repeating for several minutes. The subject was instructed to inhale when the light came on, and to exhale when the light was turned off. The subject was given a warning by intercom when recording was ready to begin, and the first light on/off cycle was then initiated and continued for a total of 40 cycles (240 sec).

Heart rate was obtained from silver/silver chloride electrodes placed on the right shoulder and lower left rib cage, with a forehead ground. The electrocardiogram was amplified by a Grass Model 12A5 amplifier. Using VPM software (19) the analog signal was constantly displayed, and the R-wave triggered a digital interrupt. The time of the R-wave was stored to the nearest msec beginning with the beat prior to light onset.

Off-line, spurious artifacts were corrected and the data were transformed to interbeat intervals (IBI, i.e., time in msec between successive R-waves). Several complementary approaches were used to evaluate changes in cardiac activity.

Two evaluations of respiratory sinus arrhythmia were conducted in which data for the first minute of recording was used for stabilization, and effects were computed over the final 30 cycles (180 sec). First, the absolute change in IBI from one beat to the next was calculated, and the mean change in successive beats across the 180 msec epoch was determined.

Second, maximum and minimum IBIs within each cycle were determined, and the difference in msec, equivalent to the range of the cycle, was then averaged across the 30 cycles. Note that for this set of computations, a one sec phase delay was introduced, since the change in rate due to a previous cycle often occurs at the beginning of the next cycle. For this measure as well as the previous measure of mean absolute change in IBI, all individual subjects' values were also converted to heart rate in beats/min.

A third series of analyses, focusing on spectral analysis of the data, was accomplished utilizing the PSPAT program (20). This program performs appropriate evaluation for stationarity of the data set. Because a minimum number of beats (200) is required for this computation, all of the IBIs were included in this analysis. Power in the spectral band of .15-.20
Hz was extracted from the PSPAT output (the respiratory rate employed in this procedure of 6 breaths/minute = .175 Hz). The natural logarithm of power in this band was used in subsequent analyses.

All measures were initially compared among groups using ANOVA, with post-hoc Newman-Keuls comparisons between paired groups reported for significance at the $p = .05$ level.

RESULTS

All of the assessments of RSA indicated significant differences among the three groups, with the solvent-exposed group consistently showing significantly reduced RSA compared to non-exposed controls. Means and standard deviations for all comparisons are presented in Table 1.

Across the final 30 cycles (180 msec), there were significant differences in the mean absolute change in IBI between successive beats ($F_{2,74} = 4.30, p = 0.017$). On this measure, the solvent-exposed subjects and anxiety patients showed nearly identical values, and both showed significant reductions in change in IBI compared to non-exposed controls. Data transformed to heart rate gave the same results ($F_{2,74} = 4.48, p < 0.015$).

Respiratory cycle range changes showed a similar pattern of effects ($F_{2,74} = 4.89, p = 0.010$). That is, the differences between slowest and fastest beats in response to respiratory activity were significantly reduced for both the solvent-exposed and anxiety groups compared to non-exposed controls (Figure 1). When recomputed on the basis of heart rates, the effects were similar ($F_{2,74} = 3.67, p = 0.030$).

Analysis of ln(Power) in the .15 to .20 Hz bandwidth also showed a significant difference among groups ($F_{2,74} = 3.56, p = 0.033$). In this case, however, only the solvent-exposed and non-exposed groups differed in post-hoc comparisons, while the difference for anxiety patients from controls did not reach significance; this may have represented a reduction of statistical
power for this measure associated with the smaller sample size for the anxiety group compared

<table>
<thead>
<tr>
<th>Variable</th>
<th>Solvent exposed</th>
<th>Anxiety disorder</th>
<th>Control (no disease)</th>
<th>Analysis of variance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. subjects</td>
<td>28</td>
<td>18</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>44.3</td>
<td>41</td>
<td>40</td>
<td>NS</td>
</tr>
<tr>
<td>SD</td>
<td>[9.9]</td>
<td>[9.2]</td>
<td>[11.3]</td>
<td></td>
</tr>
<tr>
<td>Education (years)</td>
<td>12.2</td>
<td>14.3</td>
<td>14.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>SD</td>
<td>[2.1]</td>
<td>[1.9]</td>
<td>[1.7]</td>
<td></td>
</tr>
<tr>
<td>ln(Power)</td>
<td>-3.96</td>
<td>-3.77</td>
<td>-3.01</td>
<td>0.033</td>
</tr>
<tr>
<td>.15-.20 Hz</td>
<td>[1.42]</td>
<td>[1.76]</td>
<td>[1.23]</td>
<td>S &lt; C</td>
</tr>
</tbody>
</table>

Findings based on final 30 cycles

Beat-by-beat variability

| IBI change (msec)         | 18.2            | 17.9             | 33.6                 | 0.017                    |
| SD                       | [14.2]          | [13.3]           | [31.4]               | S, A < C                 |
| Rate change (beats/min)  | 1.77            | 1.82             | 2.8                  | 0.0146                   |
| SD                       | [1.03]          | [1.01]           | [1.91]               | S, A < C                 |

Respiratory cycle changes

| IBI difference (msec)    | 74              | 74.6             | 123.4                | 0.0102                   |
| SD                       | [46.1]          | [46.2]           | [90.3]               | S, A < C                 |
| Rate difference (beats/min) | 7.56           | 7.91             | 10.69                | 0.03                     |
| SD                       | [4.15]          | [3.81]           | [5.70]               | S < C                    |
| Fast IBI (msec)          | 747.9           | 715.8            | 783.3                | NS                       |
| SD                       | [104.9]         | [116.4]          | [101.8]              |                          |
| Slow IBI (msec)          | 822             | 790.4            | 906.7                | 0.0111                   |
| SD                       | [126.8]         | [145.7]          | [145.8]              | S, A < C                 |
| Fast rate (beats/min)    | 87.4            | 91.9             | 83.3                 | NS                       |
| SD                       | [12.4]          | [15.1]           | [10.6]               |                          |
| Slow Rate (beats/min)    | 79.9            | 84               | 72.6                 | 0.0094                   |
| SD                       | [11.9]          | [16.2]           | [11.4]               | S, A > C                 |

Note: IBI = interbeat interval, S = solvent exposed, A = anxiety disorder, C = control, SD = standard deviation, NS = not significant
Figure 1. Respiratory sinus arrhythmia based on mean change in interbeat interval within respiratory cycles for the three subject groups. Exact values are provided in Table 1. Similar patterns occurred for all of the other estimates of respiratory sinus arrhythmia.

Note that the solvent-exposed and anxiety groups did not differ on any of the measures. When age and education were entered as covariates, age, but not education, was significantly associated with the measures of RSA. However, measures based on IBI were not affected: ln(Power) was reduced to a non-significant level ($p = 0.18$), and the measure of RSA based on heart rate changes was reduced to a trend level ($p = 0.058$).

To explore the group effects in more detail, separate analyses were conducted for the shortest IBIs (i.e., fastest heart rates) and longest IBIs (slowest heart rates). Although the anxiety patients appear to have the highest rates, there were no significant differences among groups for the fast rates. In marked contrast, IBIs did differ among groups for slow rates ($F_{2,74} = 4.79, p = 0.011$) with significantly longer IBIs for controls than for the other two groups, with parallel findings for heart rate ($F_{2,74} = 4.98, p = 0.0094$). This indicates that slowing of heart
rate during the respiratory cycle was significantly greater for the controls than for the patient groups, even though the groups did not differ in initial high rates.

Intercorrelations among the different measures of RSA all were high. Correlations between mean change in successive beats and mean range within respiratory cycles were high using either IBI or heart rate measures ($r = 0.98$, and $r = 0.94$, respectively, both $p < 0.001$). Correlations among the same measures and ln(Power) were somewhat lower, but still highly significant ($r$ ranging from 0.61 to 0.74, all $p < 0.001$).

Associations between RSA and the SCL-90-R Anxiety subscale were not significant for the entire group. Within the group of anxiety patients, there was a weak trend for increased reported anxiety to be associated with the fastest heart rates based on interbeat interval ($r = 0.369$, $p = .15$; this degree of association would only reach significance with at least 29 subjects). Similar trends were not present for the controls or solvent exposed groups.

For the solvent patients, rank order correlations between the Exposure Ratings were not significantly associated with any of the RSA indices.

Findings for Drug-Free Subjects: To determine the extent to which findings were independent of drug effects, we compared controls and a subset of 17 solvent and anxiety patients who were either medication free or who were receiving only drugs with no central anxiolytic, antidepressant, or blood pressure effects (e.g., antacids). With this reduced sample, RSA continued to be significantly reduced when based on beats per minute ($F_{1,46} = 4.46$, $p = 0.04$) and marginal for RSA based on interbeat interval ($F_{1,46} = 3.96$, $p = 0.053$), though differences in ln(Power) were not significant ($p = 0.15$). When the same group of 17 patients was compared with the remaining 29 patients who were receiving antidepressant, anxiolytic, or blood pressure medications, there were no significant differences on any of the measures of RSA. Similarly, no significant differences were found within either patient group examined separately for presence or absence of medication.

Long-Term Reliability: A follow-up evaluation was conducted after one year for 25 of the subjects (7 solvent-exposed, 11 anxiety, and 7 non-exposed control subjects). Table 2 shows
the test-retest reliabilities for the primary measures described above (all $r > 0.59$, all $p < 0.002$). Extremely high stability is indicated by all of the measures, suggesting that there is little change even over this relatively long period between assessments.

<table>
<thead>
<tr>
<th>Variable</th>
<th>$r$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>In[Power]</td>
<td>0.596</td>
<td>0.0017</td>
</tr>
<tr>
<td>IBI change in successive beats</td>
<td>0.676</td>
<td>0.0002</td>
</tr>
<tr>
<td>Heart rate change in successive beats</td>
<td>0.627</td>
<td>0.0008</td>
</tr>
<tr>
<td>Change in IBI range/cycle</td>
<td>0.678</td>
<td>0.00019</td>
</tr>
<tr>
<td>Change in heart rate range/cycle</td>
<td>0.694</td>
<td>0.00012</td>
</tr>
</tbody>
</table>

Note: IBI = Interbeat interval

DISCUSSION

Patients with solvent-related toxic encephalopathy exhibited decreased respiratory sinus arrhythmia as compared to non-exposed control subjects. All subjects performed an externally paced respiration paradigm with no demand for excessive respiratory volume. Patients with anxiety disorders also showed a similar pattern of reduced RSA. Findings were consistent across a variety of approaches to quantification of RSA, similar to the conclusions of Grossman et al. (13). The different estimates of RSA were highly intercorrelated, replicating previous reports
(6), although ln(Power) was less closely associated with the other measures, and was not as powerful a measure in discriminating among groups.

The differences in RSA in the patient groups appear related to a lack of slowing of heart rate during the respiratory cycle, rather than to an excessively higher rate overall. Since modulation of heart rate during the respiratory cycle has been associated with vagal control (5, 10, 14,21), the current finding is entirely consistent with prevalent notions that vagal activation is responsible for cardiac changes during respiration. In particular, the present data suggest that vagal activation is deficient in these patient groups. This is an assumption normally made in clinical studies in which decreased RSA is indicated even using deep breathing instructions (11).

Our findings in patients with clinically significant exposures to organic solvents are entirely consistent with reduced RSA function which has been reported among workers exposed to a variety of organic solvents under workplace conditions (7-10). Moreover, these effects are not likely to be attributable to medication effects: the same patterns were observed even when only patients who were free of medication or were receiving only inert antacids were compared with controls.

Typical studies employing a paced-breathing technique involve teaching a rate of breathing to the subjects, and then recording RSA during self-paced breathing during changing cognitive demands (12, 13). These procedures have an advantage in the ability to observe the effects of different tasks on RSA. The present approach, relying on an external stimulus to cue breathing, is probably not optimal for comparing within subject differences during varying processing conditions. However, the current procedure seems appropriate as a clinical evaluation technique when no separate cognitive or attentional demands are required from the subject. Moreover, this measure was highly reliable across a one-year retest period. In typical clinical settings, RSA during the Valsalva maneuver is typically employed with such other measures as the autonomic reflex screen (including quantitative sudomotor axon reflex test (QSART), orthostatic blood pressure, Valsalva ratio, and heart rate response to tile), and reflex sympathetic dystrophy (including skin temperature, resting seat output, and bilateral QSART...
measures) (22). Clinical testing for autonomic dysfunction or autonomic neuropathy involving RSA (23) is used for a variety of disorders, including diabetic autonomic neuropathy (24), ulcerative colitis (25), and cluster headache (26).

Anxiety is present both behaviorally and physiologically in toxic encephalopathy. The complex of symptomatology is similar to that seen for primary anxiety disorders. The mechanisms associated with the anxiety in both types of patient cohorts may be similar. However, data from other physiological studies (e.g., event-related brain potentials) suggests that the disorders differ in the occurrence of greater cognitive and electrophysiological impairment among the solvent patients as compared to anxiety patients (27). Thus, anxiety appears to accompany a clinically significant history of exposure to organic solvents. Given the high test-retest reliability observed over one year in this sample, the continued evaluation of respiratory sinus arrhythmia as an indicator of impaired parasympathetic function in association with solvent exposure seems highly justified.

REFERENCES


