Chem. Senses 29: 137–142, 2004 DOI: 10.1093/chemse/bjh018

Temporal Integration in Nasal Lateralization and Nasal Detection of Carbon Dioxide

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Abstract

Two experiments examined time/concentration trading for the detection of carbon dioxide, an irritant with little or no odor. Experiment 1 employed the nasal lateralization method: subjects attempted to determine which nostril received carbon dioxide and which received pure air when presented simultaneously. Experiment 2 employed a temporal, two-alternative, forced-choice, detection paradigm with monorhinal stimulation. In both experiments, stimulus duration was varied at a number of fixed concentrations to determine the shortest, detectable pulse. Under both conditions, threshold pulse duration decreased as stimulus concentration increased. Power functions with exponents of less than negative one described the data quite well: More than a twofold increase in duration was needed to compensate for a twofold decrease in concentration. Thus, for carbon dioxide, the nasal trigeminal system functions as an imperfect integrator at threshold-level.

Key words: chemesthesis, psychophysics, sensitivity, trigeminal

Introduction

Integration of stimulus energy over time allows most sensory systems, including the visual and auditory systems, to detect weaker signals than they otherwise could (Garner and Miller, 1947; Baumgardt, 1972). Accordingly, characterization of time/intensity trading is necessary for a full understanding of any sensory system. Investigators have demonstrated time/concentration trading in supra-threshold nasal irritation, as summarized recently (Hummel, 2000; Frasnelli *et al.*, 2003; Hummel *et al.*, 2003), but have done little work on integration at threshold.

Psychophysical models of integration in the detection of nasal irritation could have practical value, since occupational exposure limits are often based on irritation thresholds (Cain, 1996). Investigators have modeled the effects of molecular properties on thresholds measured in the laboratory (Abraham *et al.*, 1998; Doty and Cometto-Muñiz, 2003), but have not modeled how long subjects must be exposed to attain reasonable threshold estimates for even short-term exposure. Knowledge of the duration over which integration occurs, as well as shapes of time/concentration trading functions, for various stimuli could not only help predict the conditions under which a given stimulus will cause perceptible irritation, but might also provide insights into the physiological mechanisms underling integration (Cain, 1990).

For ammonia, ratings of intensity show almost perfect integration for durations up to ~4 s, i.e. twofold increases in duration nearly compensate for twofold decreases in concentration (Cometto-Muñiz and Cain, 1984). In the present experiments, where duration was varied to find the shortest presentation of a given concentration that would cause detectable irritation, the results of Cometto-Muñiz and Cain (1984) would predict:

$$T = KC^{(-1)}$$

where T represents threshold stimulus duration, C represents stimulus concentration and K is a constant. The equation is closely akin to some used to characterize integration in visual psychophysics (Baumgardt, 1972). Imperfect integration, e.g. from desensitization or clearance of molecules over time, could be represented by the above equation with an exponent of less than –1, whereas greater than perfect integration, perhaps from sensitization due to cumulative tissue damage from reactive irritants, could be represented by an exponent between –1 and 0. If research shows that the above model provides a good description of time/concentration trading for nasal irritation thresholds, then the model could provide a concise description of sensory function and allow characterization of full time/concentration trading

functions with measurements at a few, well-spaced concentrations.

The experiments reported below lay groundwork for future studies by examining the performance of the model outlined above using carbon dioxide (CO_2), which probably stimulates nerve endings through tissue-acidification (Hummel, 2000; Shusterman and Avila, 2003), as a model nasal irritant. Since CO_2 has little or no odor, one can potentially evaluate irritation thresholds either by detection or by nasal lateralization; the current experiments use both approaches. To determine whether the above model provides a good description of behavior will require very precise data. Such precision is difficult to achieve with large groups of subjects, but attainable with a classic approach from visual psychophysics, namely, intensive study of small groups of well-practiced subjects (Wyszecki and Stiles, 1967). The experiments below adopt this classic approach.

Experiment 1

Purpose

Experiment 1 determined minimum stimulus durations required for reliable lateralization at various concentrations of CO_2. In nasal lateralization, a spatial, two-alternative, forced-choice paradigm, subjects simultaneously receive clean air in one nostril and a chemical vapor in the other. They must determine which nostril received the chemical. Investigators commonly use this technique to measure irritation thresholds for odorous chemicals, since subjects can lateralize irritation but not odor. Recent reviews describe this technique and its history in more detail (Doty and Cometto-Muñiz, 2003; Wysocki and Wise, 2003).

Materials and methods

Apparatus

An air-dilution olfactometer (OMb6, Burghart Instruments, Wedel, Germany) presented stimuli. Devices of this type, originally designed to produce the rapid changes in concentration that studies of evoked potentials demand, have been described elsewhere (Kobal, 1985; Kobal and Hummel, 1988). Between stimulus presentations, the device delivered a steady, 5 l/min, stream of warm (38°C), humidified (97% RH) air (control flow) to each nostril. During stimulus presentation, a mixture of CO_2 and air (38°C) replaced the control flow, via vacuum switching, in one nostril, and air (same temperature and relative humidity as the control flow) replaced the control flow in the other nostril. Since switching required less than 20 ms, the output approximated square-wave changes in concentration without noticeable changes in temperature or flow. White noise, delivered through headphones, masked the noises the machine made during switching to prevent subjects from noticing trends in stimulus duration as runs progressed.

Output ports of the olfactometer consisted of 3 cm lengths of Teflon tubing (1/16″ inner diameter, 1/8″ outer) inserted into Teflon nose-pieces. The nose-pieces formed a seal at the nostril, and the bases of the nose-pieces formed a seal on the olfactometer. This ensured that all flow entered the nostrils. A flow-meter (Gillibrator 2; Gillian Instrument Corp.; Wayne, NJ) and a CO_2 monitor (GD444; CEA Instruments; Emerson, NJ) verified key stimulus parameters before each threshold measurement. Counter-timers on a multifunction data acquisition card (PCI-6023E; National Instruments; Austin, TX) provided trigger signals to the olfactometer. Custom software controlled timing and stored data.

Subjects

Two males (ages 32 and 51) and one female (age 24) provided the data. One subject was author PW, who was blind to any conditions that might cue responses trial-by-trial. The others were paid. The results will show that the data from each individual support the same general conclusion with respect to the integration function. Subjects provided consent to the IRB-approved study prior to any manipulations.

Stimuli

Compressed, medical-grade CO_2 was diluted with carbon-filtered and humidified room air. Concentration varied from 65% (6.50E+05 p.p.m.) to the lowest concentration each subject could reliably lateralize (~10%). Subjects could clearly detect concentrations higher than 65% with the briefest pulse the olfactometer could reliably produce (~50 ms).

Procedure

After placing nostrils on the nose-pieces, the subject took a breath, exhaled, and began the trial with a mouse-click. Subjects did not breathe again until after stimulus presentation, allowing flow to passively exit through the mouth. One second (s) later, the computer simultaneously triggered a 3 s signal on a monitor in front of the subject and sent a trigger signal to the olfactometer. The nostril that received CO_2 varied randomly between trials. After stimulus offset, the subject recorded the nostril into which CO_2 had been delivered (since, in previous studies, the subjects had contributed both lateralization thresholds and supra-ratings for CO_2, they were familiar with the stimulus and the sensations it can cause). Successive trials were separated by 45 s.

Within a run, concentration was fixed and pulse duration varied. For the most part, duration varied according to a two-up, one-down staircase procedure (Wetherill and Levitt, 1965), but the protocol required four consecutive correct responses before the first reversal was counted; after this, six reversals were collected. Consecutive steps changed by a factor of 1.12. For example, a 100 ms pulse would increase by 12 ms (or decrease by 11 ms), whereas a 2000 ms pulse would increase by 240 ms (or decrease by 214 ms). Runs started with stimuli ~20% longer than the best estimate of duration threshold. Initial estimates were based on 8–10

practice runs that subjects completed previously. Relatively long starting stimuli gave subjects a relatively clear sample of the target sensation at the start of each run and helped avoid spurious thresholds. Most runs required ~25–30 trials. When a subject completed more than one run in a session, at least 15 min separated successive runs. In total, subjects completed between four and six runs per concentration.

Data analysis

To further reduce the risk of spurious thresholds, only thresholds at or below the duration where subjects first achieved four consecutive correct responses counted; runs that failed to meet this criterion were repeated. Thresholds for each concentration were estimated by (i) averaging the last five reversals for each run and averaging the results across runs; and (ii) pooling data across runs and fitting cumulative Wiebull functions to plots of proportion correct versus stimulus duration (logistic and Gaussian fits yielded comparable results). Fits were made using a maximum likelihood (ML) procedure described elsewhere (Harvey, 1986). Since ML fits and averaging of reversals produced comparable threshold-estimates, only the results of averaging reversals appear below.

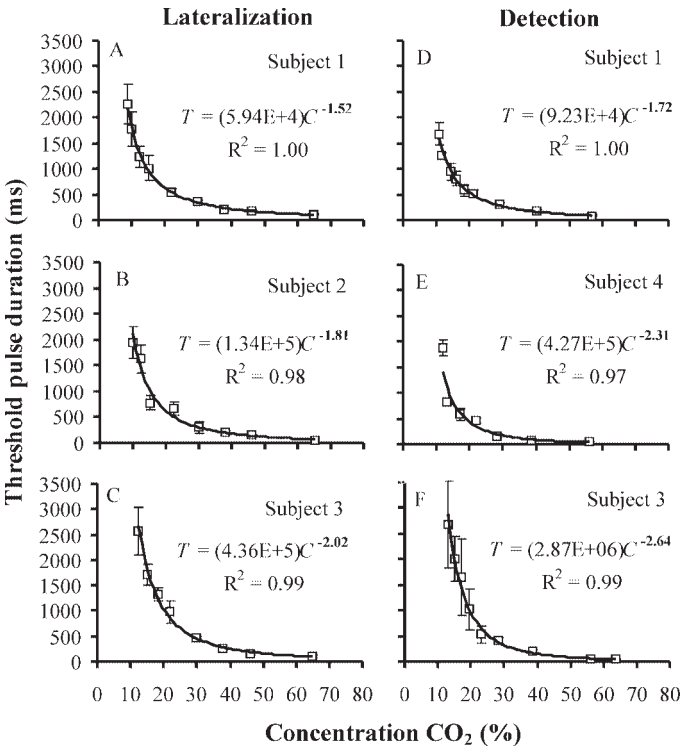

Figure 1 Threshold pulse duration as a function of concentration of CO_2. Points represent mean (across runs, ± SD) thresholds estimated by averaging reversals. The left-hand column **(A–C)** shows lateralization data. The right-hand column **(D–F)** shows detection data. Trend-lines represent power functions fit by least squares, with equations written above.

Results

We note three main features of the data (Figure 1, left-hand column of graphs). First, for all subjects, threshold pulse duration decreased as concentration increased. This finding suggests that nasal trigeminal system can integrate CO_2 over time at threshold-level. Secondly, reliable lateralization failed at concentrations below 8.8–15% CO_2, depending on the individual, even for long pulses (>5 s). The finding suggests that, with the current method and stimulus, the limit of temporal integration for the nasal trigeminal systems falls at ~2.5 s for the subjects studied. Thirdly, the power function described in the introduction describes time/concentration trading quite well (Figure 1), accounting for at least 98% of variance in thresholds. Further, the fact that functions had exponents of less than –1 (95% confidence intervals, determined from least-squares regression, were –1.46 to –1.58, –1.53 to –2.06 and –1.83 to –2.21 for subjects 1, 2 and 3, respectively) shows that integration fell short of perfection, i.e. compensation for a twofold decrease in concentration required more than a twofold increase in stimulus duration. In short, on the strength of these data, we can conclude (i) power functions provided good descriptions of time/concentration trading for this stimulus; (ii) the nasal trigeminal system functions as an imperfect integrator of CO_2 over the concentration-range studied; and (iii) temporal integration appeared complete by ~2.5 s.

Experiment 2

Purpose

Experiment 2 explored the extent to which the results of experiment 1 depended on the specific method used. Experiment 1 employed a sophisticated olfactometer, expensive both in purchasing cost and maintenance time. A simpler, less expensive solution would allow laboratories to purchase multiple devices and run subjects in parallel. Hence, experiment 2 employed a very simple device, constructed specially for the purpose. The psychophysical task also differed, in that subjects attempted to detect rather than localize CO_2. If the power model applied in experiment 1 continues to provide a good description of time/concentration trading, the result would suggest that the model might apply under a variety of conditions.

Materials and methods

Apparatus

A simple olfactometer was designed for nasal detection of CO_2 (Figure 2). It was similar in general principle to the olfactometer used in experiment 1, but, like some other devices (Lorig *et al.*, 1999), did not employ vacuum switching. The figure legend describes it in some detail to assist others who may wish to construct such a device. The device presented two 3000 ms pulses during each trial; one pulse included a dilution of CO_2, whereas the other included

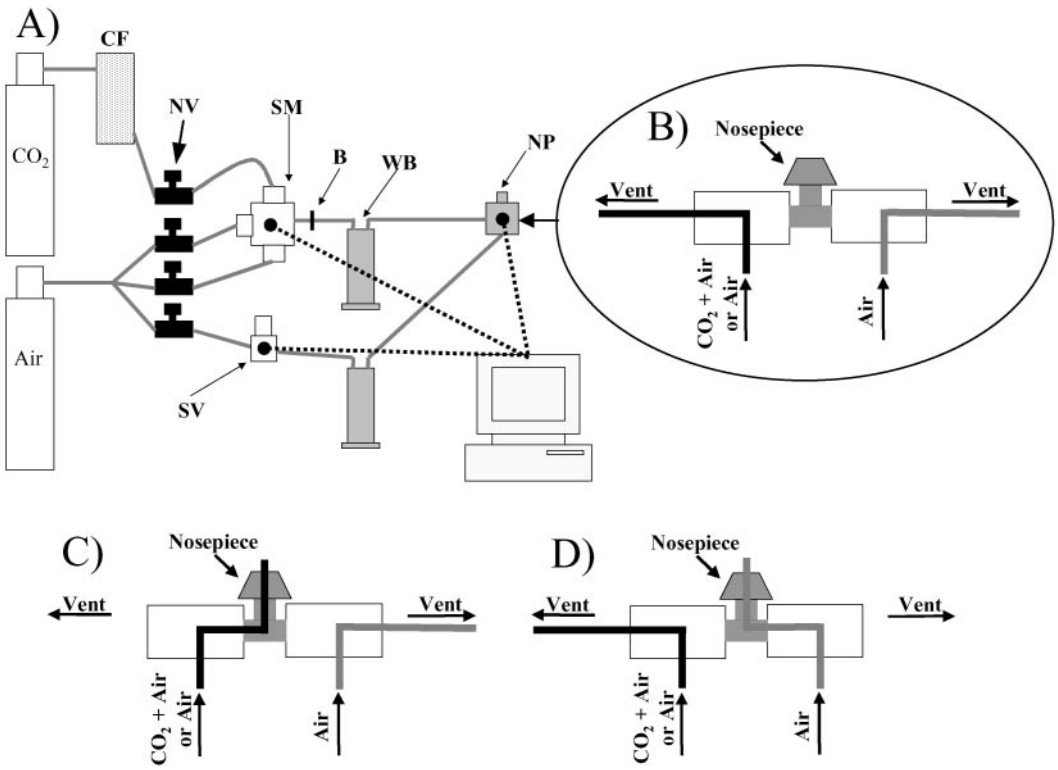

Figure 2 Compressed gasses (medical grade air and CO_2) served as sources for the olfactometer used in experiment 2 **(A)**. CO_2 passed through a carbon filter (CF) to help prevent detection of chemicals other than CO_2. Stainless steel needle valves (NV) regulated flow. A Teflon solenoid valve (SV) gated air that constituted the carrier. A three-way, Teflon solenoid manifold (SM) gated gasses that constituted the stimulus: two channels opened to produce a dilution of CO_2, or the third opened to produce a blank. A surgical steel baffle (B) created turbulence to help ensure mixing of CO_2 and air. Both carrier and stimulus passed through 250 ml gas washing bottles that contained 100 ml of distilled water to partially humidify and help regulate temperature. Needle valves, not shown, on the vent lines ensured equal back-pressure between vent-lines and nose-pieces. Finally, both carrier and stimulus passed to a nose-piece assembly (NP). The nose-piece assembly **(B)** consisted of a pair of two-way, Teflon solenoid-valves that gated flow to the nostril. For 6 s, gases were vented from the room while the system reached equilibrium pressure. Next **(C)**, one solenoid actuated to gate stimulus to the nose for a variable duration, after which **(D)** the other solenoid actuated to gate carrier to the nose for 3000 ms minus the variable duration (for a total fixed pulse-length of 3000 ms). After this, the stimulus switched from CO_2 to blank or blank to CO_2, and, after a further 6 s, the sequence depicted in sections B through D repeated.

a blank of equal duration. Each pulse in turn consisted of two phases: the first phase, of variable duration, consisted of either CO_2 or a blank; the second phase consisted of 3000 ms, minus the variable duration, of air, for a total, fixed pulse length of 3000 ms. CO_2 pulses were embedded in an air pulse to make the stimuli more comparable to those in experiment 1, i.e. pulses of CO_2 driven over the mucosa rather than various volumes injected into the nose. Flow for each pulse was set at 5 l/min at 23°C and 41% relative humidity, which was the same for both CO_2 and the blank. A rapid-response pressure transducer showed that flow reached 94% of maximum within 15 ms of nominal stimulus onset, and terminated completely within 15 ms of nominal stimulus offset. The same flow and CO_2 meters that served in experiment 1 calibrated the stimuli before each run.

Subjects

The two males from experiment 1 returned. The third subject was unavailable; another female (age 25 years) replaced her.

Procedure

Subjects first practiced velopharyngeal closure (Kobal and Hummel, 1991) until they could breathe without fogging a mirror held under the nose. Subjects placed the right nostril on a small nose-piece, forming a good seal, established closure, and started the trial with a mouse-click. Flow entered the right nostril and exited the left nostril, thereby preventing detection in the mouth and throat. After the mouse-click, a 6 s countdown appeared on the monitor, after which the subject received the first 3000 ms pulse (see apparatus). Next, another 6 s countdown appeared. During the first 3 s of the second countdown, subjects backed away from the nose-piece and breathed in through the mouth and out through the nose to help re-humidify the tissue. After this, subjects repositioned on the nose-piece and re-established closure before the next stimulus; with practice, subjects could do this with time to spare. After the second countdown, subjects received another 3000 ms pulse. The interval that received CO_2 varied randomly between trials in

a standard, temporal, two-alternative, forced-choice detection paradigm. After stimulus offset, the subject entered which interval contained CO_2. Successive trials were separated by 45 s. For the most part, other details of threshold measurement and analysis of the data matched procedures used in experiment 1.

Results

In broad terms, the results of experiments 1 and 2 agreed well. First, the lowest detectable concentrations were again ~10%, with threshold pulse durations ~2 s. Secondly, power functions with exponents less than –1 fit functions of threshold pulse duration versus concentration quite well, accounting for at least 97% of the variance in thresholds (Figure 1, right-hand column of graphs). Ninety-five percent confidence intervals of slopes were –1.61 to –1.86, –2.47 to –2.82 and –1.83 to –2.78 for subjects 1, 3 and 4, respectively. Accordingly, data from experiment 2, like those from experiment 1, are inconsistent with a perfect integrator but consistent with an imperfect integrator. In short, the model of integration applied in experiment 1 also performed well in experiment 2 in spite of substantial methodological differences.

Discussion

Differences between experiments

Subjects came closer to perfect integration in experiment 1, i.e. the slopes of threshold versus concentration functions were steeper in experiment 2 than in experiment 1 (NB slopes were steeper for subject 3 than for subject 1 in both experiments). Any number of methodological factors may be responsible for the differences between the two experiments, including the difference in psychophysical task or patterns of flow through the nose. Further, since vanilloid receptors probably play a role in transduction of tissue-acidification caused by CO_2 (Alimohammadi and Silver, 2002), and since the concentration of free protons needed to activate vanilloid receptors increases as ambient temperature decreases (Tominaga *et al.*, 1998), progressive cooling of the mucosa in experiment 2 could have decreased sensitivity to CO_2 over time. Further, the relatively low humidity of the stimulus in experiment 2 may have dried the mucosa somewhat over time, which could in turn slow absorption of CO_2 into the tissue. Future studies can explore these factors. Regardless, given the methodological differences, the agreement between experiments 1 and 2 is strong, and suggests that the power model applied might provide a good description of time/intensity trading under other nasal chemesthetic conditions as well.

Complications and limitations

In the simplest form of the power model, threshold pulse duration approaches infinity as concentration approaches zero, but lengthening duration beyond ~2.5 s did not allow subjects to detect concentrations lower than ~10% (although it is possible that a lower exponent applies at weak concentrations such that very long durations, e.g. >1 min, might have been detectable). Accordingly, an additional parameter is needed to represent the minimum detectable concentration at full temporal integration (MinC). For practical purposes, i.e. predicting the conditions under which a chemical will cause perceptible irritation, MinC probably holds the most interest. Future work that examines MinC for chemicals that vary systematically in molecular properties might suggest extensions or modifications of current models of irritant detection, such as the solvation equation of Abraham and colleagues (Abraham *et al.*, 1998).

A simple power equation might also fail for very short presentations. The visual system, for example, displays perfect integration up to ~100 ms (200 ms for rod vision) and imperfect integration up to as long as 3 s (Baumgardt, 1972). A single function described time/concentration trading in the current experiments, but we cannot rule out perfect integration for very brief pulses, since the olfactometers could not produce pulses shorter than ~50 ms.

The reader should also note that the power equation is a black box model that includes all events from entry of the stimulus into the nostril to execution of the response. For example, dynamics of concentration in the peri-receptor environment, i.e. in epithelial or sub-epithelial layers of the mucosa, will depend not only on dynamics of the generated stimulus, but also on diffusion and flow through the nasal cavity, subsequent diffusion into the tissue, and the kinetics of the reversible reaction (reviewed in Tarun *et al.*, 2003) though which CO_2 is converted to hydrogen and bicarbonate ions in the mucosa. A recently developed technique, which allows real-time tracking of pH in the mucus layer (Shusterman and Avila, 2003), may help elucidate this component of the black box. For 3 s, square-wave pulses of CO_2, the pH–time profile in the mucus layer resembled a saw-tooth, though with a more rapid onset than offset, and peaks lagged stimulus onset by ~3–13 s. Interestingly, response times in detection of CO_2 range between ~0.7 and 1.9 s (Cain and Murphy, 1980; Wise *et al.*, 2003), suggesting detection might occur before peak concentration is reached in some cases. Shusterman and Avila (2003) did not manipulate duration, but manipulations of both stimulus concentration and duration could allow researchers to plot probability of detection versus both peak and time-averaged concentration in the mucus (or perhaps other parameters such as slope of pH increase). Studies of this type can help elucidate the relationship between dynamics of peri-receptor concentration and temporal integration (assuming, of course, that other studies can determine the relationship between dynamics in the mucus layer and dynamics in the peri-receptor environment). Further, the tools of psychophysiology, e.g. combinations of psychophysics and measurement of mucosal or cortical evoked potentials (Hummel,

2000; Hummel *et al.*, 2003), may help elucidate other components of the black box.

Conclusions and directions for future research

Based on the subjects studied, the nasal trigeminal system, like other sensory systems, can detect progressively weaker stimuli by integrating over time, though the system is an imperfect integrator of CO_2. Future studies should examine larger groups of subjects and examine differences among stimuli. Cometto-Muñiz and Cain (1984) found near perfect integration for supra-threshold rated intensity of irritation from ammonia. Assuming no other differences caused the discrepancy between these results and those of the current experiments, temporal integration in the nasal trigeminal system differs either (i) between threshold and supra-threshold levels; or (ii) between ammonia and carbon dioxide. The issue can be resolved by examining temporal integration for ammonia and carbon dioxide at both threshold and supra-threshold levels. Future studies could also examine other chemicals that vary systematically in molecular properties. The molecular properties that allow more complete integration, or integration over longer periods, can provide hints as to corresponding properties of the biophase that are important for integration (Doty and Cometto-Muñiz, 2003). Radical differences between stimuli in the models that apply could prove especially interesting, as they might suggest different sets of underlying mechanisms (e.g. accumulation of molecules in the peri-receptor environment versus neural integration or cumulative damage to the tissue).

Acknowledgements

Dr Lewis O. Harvey Jr wrote the software that performed the maximum likelihood fits of psychometric functions (http://psych.colorado.edu/~lharvey/). Human subjects approval came from the institutional review board of the University of Pennsylvania. Financial support came in part from grants P50 DC00214 and T32 DC00014 from the National Institute on Deafness and other Communication Disorders and from the Eugene Garfield Foundation.

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Accepted December 13, 2003