2 Working Memory and Fear Conditioning
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Constanze Hofstötter conducted the single cue trace uninformed 0-back experiment and all of the informed experiments. She was also involved in the analysis and write up. Her contributions in the writing process made the manuscript far better than it would have been otherwise. Naotsugu Tsuchiya conducted unpublished control experiments and was also involved in the analysis and write up. Christof Koch initiated the project and secured funding. His input in the early stages of the project (while we entered a field we had no experience in) was always very useful. He also advised on analysis procedures and made substantial contributions in the write up and review processes. Experiments and analysis were conducted at the California Institute of Technology.

Here, we investigate the extent to which human classical fear conditioning depends on working memory.

2.1 Introduction
Pavlovian conditioning is widely used to study associative learning in species ranging from mollusks to flies, rodents, monkeys and humans (Baer and Fuhrer, 1982; Mackintosh, 1983; Gallistel, 1990; Thompson and Krupa, 1994; Connolly et al., 1996; Eichenbaum, 1997; Pearce et al., 1997; Tully, 1998; Squire and Kandel, 1999; Kocorowski and Helmstetter, 2001). This form of learning involves the association of an initially neutral stimulus, the conditioned stimulus (CS), with a correlated meaningful stimulus, the unconditioned stimulus (US). An unresolved question concerns the extent to
which certain forms of classical conditioning depend on higher-level cognitive processes including selective attention, working memory and awareness (Hilgard et al., 1937; Dawson and Furedy, 1976; Clark and Squire, 1998; Ohman and Soares, 1998; Carrillo et al., 2000; Knuttila et al., 2001; Lovibond and Shanks, 2002). Eye-blink conditioning is an associative learning paradigm where the role of explicit knowledge / awareness is being investigated. The paradigm involves the association of an eye-blink (a somatic motor response) with previously meaningless stimuli (CS).

Recent data showed that, in humans, associative trace conditioning of eye-blink responses requires awareness of the contingency between the CS (a tone) and the US (a puff of air to the eye), while this is not the case for delay conditioning (Clark and Squire, 1998; Clark and Squire, 1999; Manns et al., 2000b, a). In delay conditioning, the start of the US is temporally contiguous with the CS, while in trace conditioning, an interval is interposed between the end of the CS and the start of the US. Distracting subjects by having them perform a secondary task (for example, a verbal shadowing task) during a trace procedure prevents conditioning. Furthermore, subjects’ ability to report the exact nature of the CS/US relationship (e.g., “I believe the tone came before the air puff”) is greatly impaired with concurrent distraction during trace conditioning. Conversely, associative delay eye-blink conditioning appears to be insensitive to distracters. Other experiments find that both trace and delay associative differential conditioning can be disrupted by tasks that demand sufficient attention, while this is not the case for single cue conditioning paradigms (Carrillo et al., 2000; Knuttila et al., 2001). In single cue conditioning, only one CS is presented (paired with the US). In differential conditioning, two CSs are presented, one of which is correlated with US presentations (CS+), while the
other is not (CS-).

We chose fear conditioning to replicate and extend these findings with human subjects on the basis of a conditioning protocol easily extendible to mice, animals for which well established molecular tools used for manipulating genetically identifiable cell populations are available. Fear conditioning differs from eye-blink conditioning in its underlying neuronal implementation, due in part to the fact that the association involves an autonomic, rather than a somatic, motor response. Fear conditioning is easy to establish in humans and rodents, is acquired in a fraction of the trials needed for eye-blink conditioning and is tolerant to long trace periods, making it amenable to fMRI investigations (Buchel et al., 1998b; LaBar et al., 1998; Buchel et al., 1999; Knight et al., 1999). Finally, the neural circuits underlying fear conditioning, particularly the lateral nucleus of the amygdala, hippocampus and prefrontal cortex, are being vigorously explored (Fendt and Fanselow, 1999; Medina et al., 2002). We use transient elevations in skin conductance (skin conductance response or SCR) as our measure of autonomic arousal when testing responses to auditory stimuli that have been previously paired with a shock. At the same time, we distract our subjects with tasks of variable working memory load. There were parallel efforts to reproduce selected aspects of this work in mice (Han et al., 2003).

2.2 Materials and Methods

2.2.1 Equipment
Conditioning stimuli were presented and SCRs were recorded using equipment from Contact Precision Instruments (www.psylab.com), controlled by Psylab software. Silver/
Silver Chloride electrodes filled with Med Associates paste TD-246 were used for shock presentation and recording skin conductance. CS presentations were mixed into stereo headphones. Distracting tasks were written in Matlab (Mathworks) utilizing the Psychophysics Toolbox (Brainard, 1997). Analysis was carried out using programs written in Matlab as well as SPSS 10.

### 2.2.2 Subjects
Subjects were recruited from Caltech and were paid 20 dollars for their participation, based on informed consent. Their age ranged from 18-31 with a mean of 21 years. The following differential conditioning groups consisted of six subjects each: (i) delay no task, (ii) delay 1-back, (iii) delay 2-back, (iv) trace no task, (v) trace 1-back, (vi) trace 2-back. The following single cue conditioning groups consisted of four subjects each: (i) delay no task, (ii) delay 2-back, (iii) uninformed trace no task, (iv) uninformed trace 0-back, (v) uninformed trace 2-back, (vi) informed trace no task, (vii) informed trace 0-back, (viii) informed trace 2-back.

### 2.2.3 Procedure
Skin conductance electrodes were attached to the palmar surface of the first and second fingers of the non-dominant hand. Shocking electrodes were attached to the palmar surface of the third and fourth fingers of the dominant hand. Each individual’s shock level was determined using a subjective rating protocol that sought a level that was “uncomfortable but not painful”. This shock level was used throughout the experiment. After determining their shock level, subjects completed task training, the third of
three sessions of approximately five minutes each to ensure the subject had reached plateau performance. Prior to conditioning, subjects were read instructions asking them to focus on either their visual task or the wall in front of them. naïve subjects had no previous specific knowledge of the experiment except that it was a “...learning and memory experiment that involves electric shocks.” Subjects in the informed groups were read instructions that explicitly stated that an “electric shock shortly follows most presentations of a tone” and that “the tone generally predicts the occurrence of the electric shock.” They were asked to confirm verbally that they “understand that the tone usually predicts the occurrence of the electric shock.” Subjects were given a post-experimental questionnaire to assess their knowledge of the CS/US relationship (Clark and Squire, 1998) and were debriefed. The questionnaire for differential conditioning included 17 questions to assess the subject’s explicit knowledge of stimulus relationships. Subjects were not allowed to correct previous answers. The awareness index is a number between 0 and 17, corresponding to the number of correct responses. The higher the index, the more detailed the subject’s ability to recall the presence or absence of a contingency relationship between stimuli.

The informed consent procedure was reviewed and approved by the Caltech committee for the protection of human subjects.

2.2.4 Conditioning Stimuli (Figure 2-1A)

The US used in these experiments was a 0.25 second long, constant 60 Hz AC shock, the amplitude of which was determined by each subject. During differential conditioning, the CS+ and CS- were balanced between a 2 kHz tone (83 dB) and white noise (72 dB) and
were always 1 second in length. The 2 kHz tone was always used as the CS+ during single cue conditioning. During delay conditioning, reinforced CS+ presentations coterminated with the US. Reinforced CS+ presentations during trace conditioning were followed by a shock 4 seconds after the CS+ onset, leaving a 3 second trace period.

2.2.5 Experimental Phases (Figure 2-1B)
The learning procedure consisted of three phases: habituation, acquisition and extinction. In the first phase, habituation, subjects received two presentations of the CS+ and two of the CS-, in that order, to familiarize them with both stimuli. During acquisition, subjects received 24 CS+ and 24 CS- presentations, a total of 48 trials. Twenty of the 24 CS+ presentations were reinforced with a US, while four were not reinforced to allow for conditioning assessment. These four stimuli were positioned by randomly removing the US following one of the six CS+ presentations in each of the four blocks of 12 trials (six CS+, six CS-) during acquisition (excluding the first two CS+/US pairings in the experiment). During the extinction phase, subjects received twelve nonreinforced CS+ and twelve CS- presentations. CS+/CS- presentations occurred in random order with the limiting factors being a) that no more than two presentations of a specific CS occurred in a row and b) six of each occurred in each block of twelve trials. Intertrial intervals were uniformly distributed from 15-25 seconds.

Single cue conditioning experiments were performed in a similar fashion using a phantom CS-, a marked period of time that had no actual stimuli instead of an explicitly unpaired stimulus. The analysis protocol for single cue conditioning was analogous to the differential protocol using these phantom CSs-. When compared to a US only control
method, our procedure has the disadvantage of not controlling for unassociated stimulus SCR; however, it also has several advantages. It allows a comparison within subjects, a more effective means of detecting conditioning. This method also avoids the pitfalls of using a US only protocol where the US/CS- relationship is randomized or explicitly unpaired. The former may be associated with elevated CS- responses due to a generally elevated anxiety level. The latter tests the subject’s ability to learn the anticorrelated relationship between the CS and US to enable suppression of the aforementioned general anxiety. It should be noted that our results show that working memory tasks interfere with our single cue trace conditioning protocol, adding validity to the idea that using the phantom CS- allows for accurate and sensitive detection of conditioning.

2.2.6 Distracting Tasks (Figure 2-1C)
To confirm that the conditioning protocols were effective, one group of subjects was excluded from performing a task (i.e. for each procedure they simply stared at the wall). The degree to which conditioning depends on working memory was assessed by asking a group of subjects to perform an n-back memory task during a conditioning procedure. Subjects had to press a key every time a given number appeared (0-back), when the present number matched the one before it (1-back), or whenever it was identical to the one before the previous (2-back). Only single cue trace subjects were asked to perform the 0-back task. The 0-back task involves the same input and the same motor output, including frequency of response, as the 1 and 2-back tasks, but is only minimally dependent on working memory.

The numeral 1, 2, 3 or 4 appeared at a constant rate that, for a 2-back task, was
adjusted for each subject to achieve a performance of approximately 85%. The mean rate of 2-back presentation was 1 Hz for differential subjects (88% correct), 1.33 Hz for uninformed single cue subjects (84% correct), and 1.2 Hz for informed single cue subjects (85% correct). All 1-back and 0-back tasks were performed at a presentation rate of 1.33 Hz. The mean performance for subjects focusing on the 1-back task was 93.5%. The mean task performance for single cue subjects in the 0-back group was 98% for uninformed subjects and 99% for informed subjects.

2.2.7 Analysis of SCR
A skin conductance response was measured as the maximal amplitude difference of more than 10 nS that occurred in a 1 to 4 second window after the delay CS onset, or in a 1 to 7 second window following the trace CS onset. Valid responses were range corrected by the largest amplitude response for each subject (Lykken, 1972). When there was no response, a zero-amplitude response was included in the analysis.

Habituation analysis was performed for differential conditioning using a paired t-test and a normalized ANOVA. No significant SCR differences were observed between the CSs, with one exception. Only the differential delay group performing no task showed an SCR difference (p<0.05) using the normalized ANOVA. However, no difference was observed using the paired t-test. The discrepancy between these statistical tests, the robustness of the conditioning for this group and the biased presentation order of the CSs lead us to regard this difference as inconsequential.

All CS+ presentations were compared to adjacent CS- presentations. During acquisition, when there were two adjacent CS- presentations available for comparison to a CS+, one was chosen at random.
Reported ‘p’ values for conditioning were ranked F-statistics for bootstrapped ANOVAs (10^5 re-samples per test). Four other tests were performed for confirmation: ranked F-statistics for a permuted ANOVA (10^5 re-samples); a square root corrected ANOVA; a permutation test (Efron and Tibshirani, 1998) (10^5 re-samples); and a paired t-test (averaging each trial across subjects). These confirmation statistics yielded similar results, with the exceptions noted below. Differential awareness correlations used a least squares fit. Analysis of main factors and interactions were performed using the GLM univariate ANOVA in SPSS (v10, Macintosh). These tests utilized the mean CS+, CS- difference for each subject.

2.2.8 Trial Effects
Trial effects were analyzed overall for acquisition and extinction phases of the experiment to assess the possible presence of consistent trends, such as a gamblers fallacy effect. Whether or not conditioning has occurred is assessed by comparing the results of the habituation analysis to the results of the acquisition/extinction phases of the experiment. In general, no CS+/CS- (or phantom CS-) difference is present during habituation. There is a significant difference (p<0.05) between CS+ and CS- (or phantom CS-) responses during acquisition and extinction when conditioning has occurred. Learning is then assessed by the presence of this difference (reported in the results below).
2.3 Results

2.3.1 Differential Conditioning

No task  Differential conditioning relationships were first established for trace and delay paradigms, using six subjects per group who were not asked to perform any task during conditioning. The delay group (Figure 2-2A) shows larger SCRs to the CS+ test trials than to adjacent CS- presentations (p<0.001). The same is true of SCRs during trace conditioning (Figure 2-2B, p<0.001 paired t-test p<0.01). No significant trial effects are present in either group. Thus, trace and delay differential protocols are sufficient to produce conditioning when performed alone, without distraction.

Concurrent distracting task  The n-back working memory task served as a distraction from the concurrently performed conditioning protocol. When six subjects performed the 1-back working memory task during differential delay conditioning (Figure 2-2C), there is a statistically significant difference between responses to CS+ and CS- during conditioning (p<0.01). However, when a 1-back working memory task was performed by six subjects during differential trace conditioning, there is no significant difference between SCRs to CS+ and SCRs to CS- (Figure 2-2D). When subjects carried out the 2-back task, there is no significant difference between responses to CS+ and CS- for either delay (n=6) or trace (n=6) conditioning (Figure 2-2E, F). No significant trial effects are present.

Differential main effects  A univariate ANOVA using the mean CS+/CS- differences for each subject showed that both the delay/trace difference and task level were significant main effects (p < 0.05 and p < 0.01, respectively). The delay/trace by task interaction was not significant, but may have been lost in the floor effect between differential trace 1-back and differential trace 2-back.
2.3.2 Awareness of CS/US Contingency

Correlations between awareness and CS+/CS- amplitude differences

There is a positive correlation between the awareness index and strength of conditioning (mean [CS+ - CS-]) during extinction for the 18 subjects carrying out the differential trace learning procedure (Figure 2-3). The correlation has an adjusted $r^2$ value of 0.334 (Pearson coeff. = 0.611, p < 0.01). No significant correlations between contingency awareness and CS+/CS- difference are present for trace acquisition, or for either acquisition or extinction during delay conditioning.

Differential conditioning task interference

The twelve subjects who were not performing a task during differential conditioning (six delay, six trace) have an average awareness index of 15.2 (maximum 17). Twenty-four subjects who were performing a task during differential conditioning (trace and delay, 1-back and 2-back, six subjects in each combination of conditions) have an average index of 13.4. A univariate ANOVA utilizing the awareness questionnaire score to test factors that influence awareness show significant main effects for both task (p<0.05) and delay/trace (delay mean = 14.8, trace mean = 13.2, p<0.05) with no significant interaction. In summary, both the addition of a task and the addition of a short trace interval reduce the subject’s ability to report the CS/US contingency relationship in a post-experimental questionnaire.

2.3.3 Single Cue Conditioning

No task

Single cue conditioning relationships were established in a group of four delay subjects and four trace subjects who did not perform any distracting task during the conditioning protocol. Both groups (Figure 2-4A and B respectively, n=4 each) show
significant differences between CS+ test trials and adjacent phantom CS- presentations (p<0.001). No significant trial effects are present.

**Concurrent distracting task** A group of four single cue delay subjects and a group of four single cue trace subjects were asked to focus on the 2-back working memory task during conditioning (Figure 2-4C and D respectively). The subjects that carried out the 2-back task during single cue delay conditioning show greater SCRs to CS+ test trials than to phantom CS- trials (p<0.001). The 4 subjects performing the same 2-back task during a trace conditioning protocol show no significant conditioning for the experiment. No significant trial effects are present. While the 2-back task interferes with single cue trace and differential delay conditioning (Figure 2-2E), there is still a significant CS difference in single cue delay conditioning during the 2-back task.

**Uninformed 0-back task** A group of four subjects had to signal whenever a particular number appeared on the screen (0-back) during the single cue trace conditioning procedure (Figure 2-5A). There is no statistically significant difference between responses to the CS+ and the phantom CS- for this group. No significant trial effects are present. Although the 0-back task is a simple signal-detection task, there is no significant CS difference during single cue trace conditioning.

**Informed subjects** For the group of four informed subjects not distracted by any additional task (Figure 2-5B), and for the four performing the 0-back task (Figure 2-5C), there are significant differences between responses to the CS+ and the phantom CS- during single cue trace conditioning (p<0.001). However, for the group of four informed subjects performing the 2-back task (Figure 2-5D), there are no significant differences between responses to the CS+ and the phantom CS-. No significant trial effects are
present in any group. Prior explicit knowledge of the stimulus contingency facilitates, but does not guarantee, single cue trace conditioning.

2.4 Discussion
It is generally held in both eye-blink and fear conditioning that acquired trace and delay CS/US associations are distinct forms of learning. While the key difference between the two is the interposition of a temporal gap between the end of the CS and the start of the US, they involve different neural circuits and obey different regularities. For instance, acquisition of trace but not delay conditioning is critically dependent on hippocampus and certain prefrontal structures (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Maren et al., 1997; Weible et al., 2000; McLaughlin et al., 2002; Quinn et al., 2002). In addition, Clark and Squire (Clark and Squire, 1998) showed that differential trace eye blink conditioning depends on CS/US contingency awareness, while this is not the case for delay conditioning (see also (Manns et al., 2000a; Clark et al., 2001; Manns et al., 2002)). This claim has been challenged. For example, Carrillo, Gabrieli and Disterhoft (Carrillo et al., 2000) demonstrated that not only single cue delay, but also single cue trace conditioning, was unaffected by division of attention. They used a dual-task paradigm to study the ability of subjects to acquire eye blink conditioning while their attention is concurrently engaged by watching a silent movie or verbal shadowing. Differential delay conditioning is, however, affected by the division of attention. Therefore, Carrillo and colleagues argue that the additional attentional demands imposed by the need to discriminate CS+ from CS- prevent delay conditioning from occurring when subjects have to perform a second task (see also (Mayer and Ross, 1969; Knutinen
et al., 2001), and above results).

In this paper, we present experiments on fear conditioning. Fear conditioning differs from eye-blink conditioning in that it is dependent on the amygdala for both delay and trace conditioning, while eye-blink conditioning shows a similar pattern of dependence on the cerebellum (Medina et al., 2002). Our experimental paradigm involves association between tones or noises as CSs and electric shocks as USs. As a measure of autonomic conditioning, we utilize increases in skin conductance in a comparatively young population (college students). We choose fear conditioning since it can easily be adapted to rodents, allowing the use of molecular and genetic tools to study the underlying neuronal substrates of conditioning.

The general pattern of our findings is that the extent of associative autonomic conditioning depends on the cognitive load involved. The larger the demand on the system, the less conditioning occurs. We use the mean CS+, CS- difference for each group as a measure of strength of conditioning. This measure of conditioning is plotted in Figure 2-6 for each of our experiments. Figure 2-6 A, B, and C represent the transition from uninformed differential (A) to uninformed single cue (B, removing the second anticorrelated CS) and then the addition of explicit knowledge of the CS+/US relationship in the informed single cue condition (C). Task difficulty increases from left to right on the horizontal axis. The axis into the plane of the paper separates the trace and delay groups by the stimulus onset asynchrony (SOA) between the CS+ and US (Trace SOA = 4 sec, Delay SOA = 0.75 sec). Moving in Figure 2-6 from bottom to top (panel C to A), from right to left, or out of the plane of the paper all result in an increase in overall conditioning complexity for the subject. A decrease in conditioning with any difference
from the simplest protocol supports the hypothesis that as conditioning complexity increases, the amplitude/probability of conditioning decreases. This is reflected in a univariate ANOVA where the main effects single/differential, delay/trace, task level, and informed/uninformed effects are all significant. The only significant interaction is between single/differential and delay/trace. The lack of a significant delay/trace task effect could be due to a floor effect, because the conditioning amplitude has reached zero for trace conditioning protocols in the first level, where a concurrent task has been added. We are not making any claims about the uniqueness of this representation. Others are possible and might prove advantageous.

It should be noted that Figure 2-6 is compatible with the existence of secondary tasks that do not interfere with trace conditioning in naïve subjects. A similar plot might also prove beneficial in summarizing the eye blink conditioning literature.

In Figure 2-6, there are several interesting points to note. First, similar to results shown by others in eye-blink conditioning (Mayer and Ross, 1969; Carrillo et al., 2000; Knuttinen et al., 2001), differential delay conditioning is susceptible to interference tasks. Second, it should be noted that although reduced, single cue delay conditioning still occurred during the difficult 2-back task. Third, all of the distracting tasks tested so far interfere with the trace fear conditioning protocol in our naïve subject pool. This is even the case for the 0-back task under single cue trace conditioning, a simple signal detection task—pressing a button whenever the target appeared in a string of numbers—with minimal attentional and working memory demands (subjects only had to remember a single target number during the 20 minute conditioning procedure). Fourth, it is only when we briefed subjects ahead of time about the nature of the experiment that we could
reliably induce trace conditioning under a 0-back task. We conjecture that this focused their attention onto the CS/US relationship and boosted learning.

The evaluation of the post-experimental questionnaire showed a correlation ($r^2=0.395$) between differential trace subjects’ awareness scores and conditioning during the extinction phase. We found no significant correlation in the acquisition phase, nor did we find a correlation for either phase of delay conditioning. The correlation found establishes a link between explicit knowledge of the CS/US relationship and the expression of trace fear conditioning during extinction. It is different from the explicit knowledge/conditioning correlations reported in (Clark and Squire, 1998), because our correlation occurs in fear conditioning and is true for the extinction phase as opposed to acquisition. A challenge for the future will be to develop on-line measures of CS/US contingency awareness (LaBar and Disterhoft, 1998; Lovibond and Shanks, 2002).

One might expect that subjects who are aware of the stimulus contingency would show a gambler’s fallacy effect where the differential response amplitude during extinction phase increases for a number of extinction trials. Such a pattern was reported during eye blink conditioning (Clark et al., 2001). We failed to find any significant trend in response slope. In fact, it is likely that if higher awareness scores cause stronger conditioning, this may lead to more than one response strategy (for example, higher initial responses with rapid extinction or gambler’s fallacy). Our results also show a reduction in awareness in those groups who were performing a task compared to the no task controls.

Two possible non-exclusive explanations for our results are the following. One, explicit knowledge of the CS/US relationship is necessary for the expression of more
complex types of conditioning. When that explicit knowledge cannot be acquired, conditioning cannot be established. This is supported by the fact that task performance reduces both the awareness index and the efficacy of differential conditioning. In addition, explicit prior knowledge of the CS/US relationship compensates for some of the interference in single cue trace conditioning caused by concurrent task performance. Two, it is possible that concurrent task performance suppresses amygdala activity and subsequently suppresses the establishment of a conditioned fear response. Medial prefrontal cortex stimulation in rodents shows suppression of the basolateral complex of the amygdala (Rosenkranz and Grace, 2001). Furthermore, the n-back task shows an increased fMRI BOLD signal in human prefrontal areas that could be linked to suppression of normal brain activity under adverse conditions (Pochon et al., 2002). Either of these observations could explain fear conditioning interference by concurrent task performance.

We find it surprising that the working memory task has such a strong effect on both delay and trace conditioning. In the next chapter, we seek to identify areas of the brain that correlate with explicit and implicit learning during conditioning using fMRI. We hypothesize that the brain areas that correlate with explicit learning will be in the same prefrontal regions that are active during working memory tasks.
2.5 Figures and Legends

Figure 2-1

A. Conditioning Stimuli

- Delay conditioning consisted of a 0.25 second long electric shock that overlapped and co-terminated with the 1 second long CS+ (tone or noise).
- Trace conditioning, the CS+ was followed 3 second later by the US.

B. Experimental Phases

<table>
<thead>
<tr>
<th>Habituation</th>
<th>Acquisition</th>
<th>Extinction</th>
</tr>
</thead>
<tbody>
<tr>
<td>2CS+, 2CS−</td>
<td>20CS+/US, 24CS−, 4CS+ only</td>
<td>12CS+, 12CS−</td>
</tr>
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C. Tasks

- 2-back: X
- 1-back: X
- 0-back: X

Figure 2-1 A) Delay conditioning consisted of a 0.25 second long electric shock that overlapped and co-terminated with the 1 second long CS+ (tone or noise). In trace conditioning, the CS+ was followed 3 second later by the US. B) The conditioning protocol consisted of three phases (habituation, acquisition and extinction). C) Distraction tasks and conditioning procedures were performed concurrently. During a 0-back task, the subject pressed a key (marked by an X) whenever a predetermined number appeared (4 in this case). During a 1 or 2-back task the subject pressed a key whenever the number matched the one before it or the one before the previous one, respectively.
Figure 2-2

Figure 2-2 Mean range corrected SCRs to CS presentations for each trial. Thirty-six subjects (6 per group) participated in either the differential delay (A, C or E) or trace (B, D, or F) learning procedure without any task or while being distracted by a 1-back or a 2-back task. Mean range corrected SCRs to CS+ are shown in solid lines with cross markers. Mean range corrected SCRs to CS- are indicated by dashed lines with circles. Significant conditioning exists during the delay procedure with no concurrent task and while performing the 1-back task. Only under no task conditions did we find significant trace conditioning. The vertical line marks the last test trial during the acquisition phase.
Figure 2-3 Scatter plot of mean range corrected differences between CS+ and CS- and the subject’s awareness index. During differential trace extinction (Fig. 2B, D, and F; trial 5-16) subjects show a linearly increasing relationship between average amplitude of response difference and post-experimental questionnaire score (adjusted r² = 0.334, Pearson coeff. = 0.611, p < 0.01, n= 18). Subjects show no significant correlation between conditioning (average range corrected CS+ -CS-) and awareness index during differential trace acquisition, differential delay acquisition or differential delay extinction.
Figure 2-4 Mean range corrected SCRs to CS presentations for each trial. Sixteen subjects (4 per group) participated in either single cue delay (A or C) or trace (B or D) conditioning without any distraction or while carrying out a 2-back task. Mean range corrected SCRs to CS+ are shown in solid lines with cross markers. Mean range corrected SCRs to marked phantom CS- time points are indicated by dashed lines with circles. Significant conditioning exists for delay conditioning with no concurrent task and while performing the 2-back task. Significant trace conditioning is present only while no task was performed. The vertical line marks the last test trial presented during acquisition.
Figure 2-5 Mean range corrected SCRs to CS presentations for each trial. Sixteen subjects (4 per group) participated in either informed or uninformed single cue trace conditioning without being distracted (no task), or while carrying out a 0-back or a 2-back task. Mean range corrected SCRs to CS+ are shown in solid lines with cross markers. Mean range corrected SCRs to marked phantom CS- time points are indicated by dashed lines with circles. Significant conditioning is present for informed trace conditioning while subjects performed no task or a 0-back task. Significant uninformed trace conditioning is only present without a concurrent task (Figure 2-2B). The vertical line marks the last test trial presented during acquisition.
Figure 2-6 Summary of our data plotted in a 3-D space capturing the contingencies of our protocol. The vertical axis marks the group average for each subject’s average range corrected and normalized CS+, CS- difference. The horizontal axis marks the task difficulty. The axis into the plane of the paper marks the group as trace or delay using the difference in CS/US onset (SOA) in seconds. In addition, the line for trace is hatched while the line for the delay group is solid. 

"**" indicates significant conditioning at p<0.01. Areas of the lines that are not filled in are meant to assist the stability of the figure, not to imply any prediction about the magnitude of conditioning in that area.

A) Mean group differences for differential subjects. B) Mean group differences for uninformed single cue subjects. C) Mean group differences for single cue informed subjects. Our results indicate that the higher the cognitive load, the smaller the CS+/CS- difference.