

A system for quantitative analysis of associative learning. Part 2. Real-time software for MS-DOS microcomputers

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Abstract

Microcomputer software was designed and used to control the timing and delivery of sensory stimuli and to acquire and analyze behavioral data during classical conditioning experiments. The software package runs under DOS 4.x through 6.x (earlier versions run under DOS 3.x) on PC AT-compatible microcomputers coupled with appropriate interface hardware (see Thompson et al., 1994). The software controls timed delivery of up to 8 conditional stimuli. It can collect behavioral data from 2 subjects simultaneously performing the same task (e.g., eyeblink responses) or from a single subject performing 2 different tasks (e.g., both eyeblink and conditional discrimination tasks), permitting its use in a number of experimental paradigms. Digital timing signals are adjustable for different stimulus output systems. Behavior is continuously monitored onscreen, ensuring consistent measurement across trials. Real-time performance measures of the presence or absence of conditioned responses allow coordination with external events (e.g., serum sampling, drug delivery, or single-unit recording). Quantitative measures are generated both for each trial and for complete sessions. Records are stored to disk and can be printed or merged for statistical analyses. Data can be archived on standard media, and internal software utilities translate files for export to PC and Macintosh™ programs. This system and the hardware described in the preceding paper combine ease of use with extremely replicable behavioral measurements across trials, sessions, subjects, cohorts, and studies.

Keywords: Eyeblink conditioning; Nictitating membrane response; Associative learning; Microcomputer software; Stimulus timing; Behavioral response analysis

1. Introduction

The ideal data collection software must meet multiple and rigorous requirements for use in an experimental setting. It must be flexible, to allow for more than one mode of use. It must be extensible, to allow for the unforeseen needs and changes in experimental design that inevitably occur. It should have multiple uses, to eliminate the need to learn and use different software packages for slightly different tasks. It should be easy to use, comprehensive, comprehensible, and above all reliable. Few experimenters will tolerate loss of data due to software bugs or crashes. Several pieces of

software designed to run on earlier computer hardware or offering desirable features for use in learning and memory studies have been described in the past (Solomon and Babcock, 1979; Scandrett and Gormezano, 1980; Lavond and Steinmetz, 1989). The software package described below exemplifies one programmer's attempts to more fully meet the challenges listed above, and to provide reliable control of stimulus timing as well. The software in actual operation has proven extremely robust and comprehensive, allowing nearly continuous daily use in our laboratory for the past 5 years without data loss due to software failure.

The NM500 software system was written entirely in Microsoft Quick Basic Version 4.0 and Microsoft Assembler. It was designed to provide modular, multipurpose routines to control classical conditioning experiments. To allow modification for use in different experimental situations, NM500 (the fifth generation of this nictitating membrane (NM) experimental software) was

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written in BASIC, a popular microcomputer language that is relatively easy to read, modify, or debug. The system was not compiled, allowing program execution without linking to external libraries. Quick BASIC was selected for its superior use of list editing and structured commands, as well as its ability to present a consistent end-user interface. Since interpreted BASIC is not suited to real-time sampling and analysis of data, assembler subroutines are called from the BASIC program for low-level time-critical functions. The completed software achieves a very satisfactory balance between the required performance requirements and the design goals of consistency, flexibility, and ease of operation. NM500's performance is robust on 80286-based microcomputers (the original development environment), while non-critical operations (such as menu selection) are significantly improved on 80386 or better machines.

2. Methods and Use

Behavioral data is sampled through 2 of the 8 analog input bits on an analog-to-digital (A/D) board installed on the PC bus, allowing simultaneous recording and analysis of 1 behavior from each of 2 subjects or 2 behaviors from 1 subject (see companion paper: Thompson et al. (1994) for a full explication of a

hardware interface designed for use with this software). The main training screen (Fig. 1) displays continuous as well as trial-by-trial behavioral graphics in color (reduced to black and white for reproduction here), with accompanying numeric analyses of performance. The software eliminates the need to use a storage oscilloscope to monitor analog or digitized data from conditioning trials, as the computer functions as a digital oscilloscope. Behavior is analyzed on-line on a trial-by-trial basis, providing instantaneous displays of raw data, automated scoring of conditional responses (CR), and cumulative performance records across the training session. Both CR and unconditioned responses (UR) are analyzed and stored on the computer's hard disk drive. Summary tables (.TBL files) are generated and stored for later analysis, and binary records of behavior within trials (.BEV files) are stored for later visual analysis, printing, archival use, or re-calculation of statistical data. Results can be transferred to other systems as binary numeric, ASCII text, or image (graphic) data files. Both numeric and graphic files are routinely off-loaded from this system for final summary on Macintosh™ computers. The NM500 system can produce files in formats compatible with word processors, spreadsheets, and statistical packages on a number of other popular microcomputers.

The human interface is designed for user friendliness and structured for ease of use. Most operations of

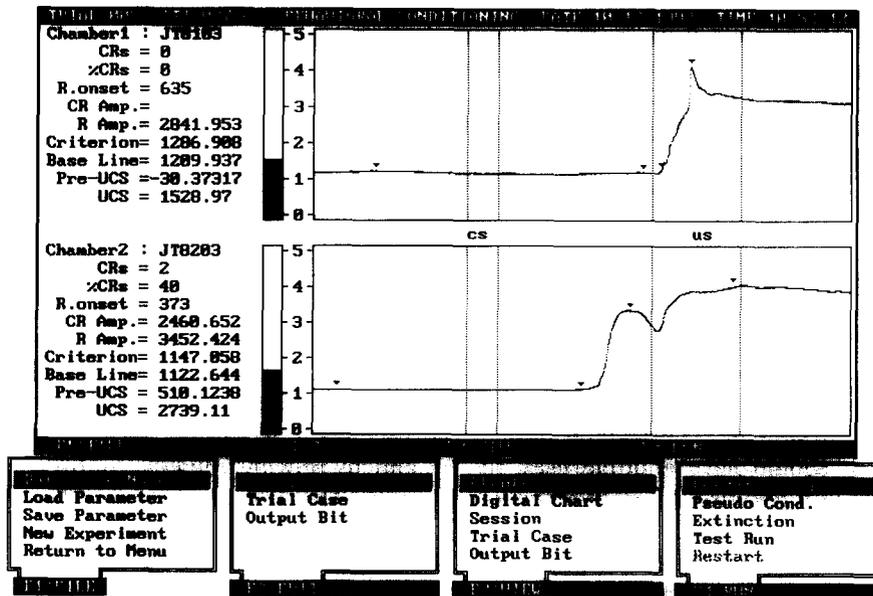


Fig. 1. The inner conditioning shell of NM500 in actual use. Conditioning variables and trial execution are controlled by the function keys below. Nictitating membrane responses from 2 rabbits are shown. The rabbit in the top chamber exhibits an UR, while the rabbit in the bottom chamber exhibits a CR preceding the UR within the trace ISI (500 ms in this example). Behavioral data is analyzed on-line, with digital read-outs at left detailing performance up to and including the current trial. An ITI clock counts down to the next trial. Four small arrows on the behavioral plots indicate response amplitude events used in the analysis and scoring of responses. The arrows appearing in the pre-CS period indicate the peak baseline amplitude, while arrows within the ISI indicate the first sample equalling this peak (both traces) and the first sample exceeding the criterion defining a CR (bottom trace only). The third arrow on the top trace indicates that the first response occurred within the US interval, while the final arrow on both traces indicate the peak UR amplitude.

NM500 consist of selections from pop-up menus. Menu selections can be made using function keys, keypad arrow keys, or by typing the initial letters of the menus displayed. Multiple menu selection methods were incorporated into the program, to allow each operator to adopt a menu selection mode with which he/she is most comfortable. When an incorrect menu selection is made, the operator can cancel it and return to the previous menu selection by pressing the escape key. In dialog boxes, default prompts are frequently indicated (e.g., directories and subdirectories for file storage), but can be supplanted manually (pressing the home key clears the text entry box for user input). In case of an operator error (e.g., a file name is entered which already exists) the program displays an alarm dialog box, and information instructs the user how to avoid repeating the error.

In our experiments, 1 or 2 channels of raw analog data are generally sampled at a 1 kHz sampling rate, but the software allows simultaneous sampling of 2 behavioral input channels at rates up to 10 kHz. At this high sampling rate, using 2-s trials and 80 trials per session, the data files collected total more than 3 Mbytes per subject per session. In normal use, with a sampling rate of 1 kHz (which gives extremely good resolution of eyeblink responses; see Fig. 1), the data files generated for a single subject's session total slightly

less than 300 kbytes, allowing 4 complete days of training records for a subject to be stored on a single high-density floppy disk.

In NM500, inputs from the A/D board are treated by the BASIC program as strings, with no conversion to numeric formats. Data saved to disk are also represented as string expressions, since conversion can take longer than reading or writing the data to disk, especially when a large number of samples is involved. As a consequence, reading or writing an entire training session's data for analysis is very fast, even though the shell program is written in interpreted BASIC. The NM500 BASIC program shell passes string data to a machine language subroutine that converts it to numeric data as needed.

An important feature of this system is the use of a consistent file structure to identify the training paradigms used. Within 1 conditioning session, 1 or more trial types can be repeated at random. The.INF file, detailed later, preserves a record of the trial type (e.g., conditioned stimulus (CS) alone; CS-US paired; unconditioned stimulus (US) alone; CS₁ followed after an interstimulus interval (ISI) by CS₂; CS⁺ paired with the US and CS⁻ unpaired; etc.), which avoids duplication of information in data files. Analytic routines can read the information file to review the whole session, then efficiently read via 'random access' only the raw

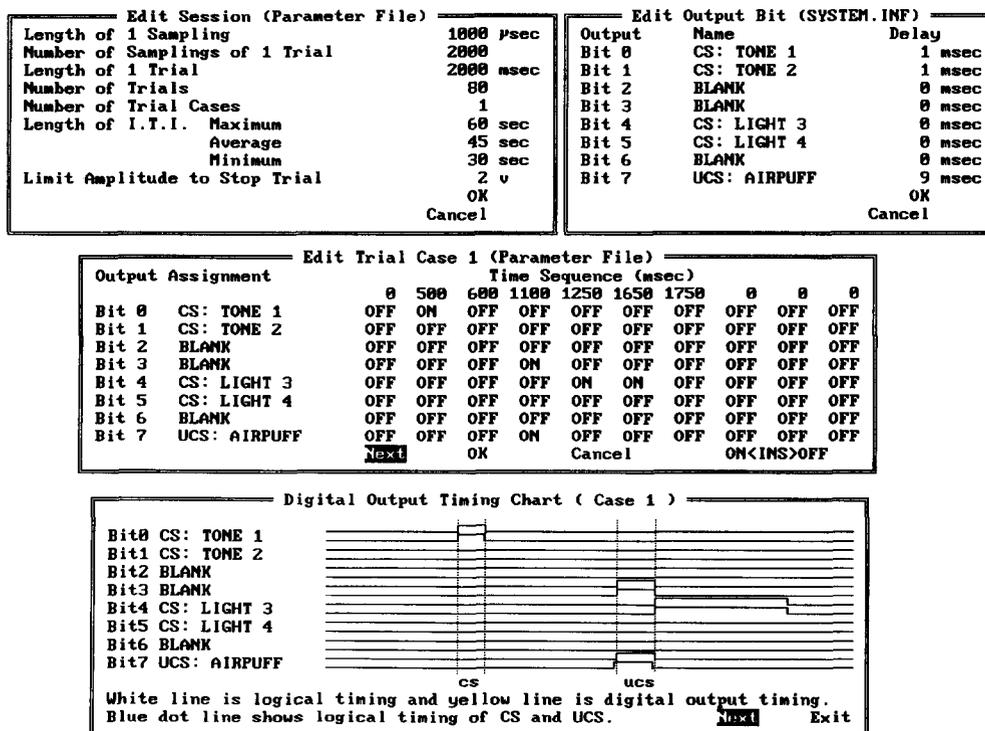


Fig. 2. Screen dumps of the controls provided for customizing behavioral experiments. At top left, variables controlling sampling rate, trials, and intertrial intervals are set. At top right, the assignments of individual bits on the digital output of the A/D converter are made. In the middle panel, an example of a trial case timing delivery of tone and light CSs and an airpuff US; up to 10 separate trial cases can be defined for each session. At bottom, the adjustment of timing signals to allow for measured lags in output hardware responses is illustrated; 2 lines for each of the 8 output bits illustrate actual stimulus timing and the digital signals required to achieve these timing relationships.

data from the trials of the specific type required for analysis. This is important when a conditioning session consists of more than 1 type of conditioning trial, as post-session analyses need to summarize data from non-sequential trials (e.g., CS or US trials in pseudo-conditioning; or CS⁺ or CS⁻ trials in discrimination conditioning). This information is also used for later analyses, such as comparisons between groups.

As described below, conditioning sessions can be initiated or replayed; statistical data and behavioral responses can be analyzed, printed, or saved in other file formats; and the specific interface hardware used can be adjusted for by NM500. The program is modular, with the inner shell (Fig. 1) containing the main training screen that controls experimental stimuli, trial and session set-up utilities, and data collection. An outer shell contains analytic and system level utilities. The inner and outer shell functions are described below. As noted, in most instances user initiated operations can be interrupted by pressing the escape key.

2.1. Inner (main conditioning) shell

This portion of the user interface (see Fig. 1) offers full controls for setting up different experimental paradigms, specifying stimulus timing relationships, and running experiments, with on-line behavioral displays and performance analyses.

2.1.1. F1: CONDITIONING

These commands allow the user to assign names for storage of the data collected on each of the 2 input channels (one or both may be used), to assign or save trial and session parameters (described below), to initiate a new session, or to exit the conditioning shell to the outer utility shell. Commands are briefly described below.

Data file name. By convention, and due to the limitations of DOS file names, subjects are assigned a 1- or 2-letter and 2- or 3-digit code (e.g., JT297) designating their experimental identification, with an additional 2 digits of the file name designating the session number (i.e., JT29707 designates the data for subject JT297's seventh training session). The default directory for storage of all raw data files is '\EXP_DATA'.

Load parameter file // Save parameter file. Parameter files specify a large number of variables controlling stimulus presentation and data collection (see Fig. 2). Each parameter is fully editable, using F2 in this inner shell of NM500. New parameter files can be created from scratch or saved for re-use, facilitating consistent application of the same paradigm across sessions. The sampling rate and length of each trial, as well as the number of trials per session, determine the size of the

data files generated. The types of trials presented (trial cases) can be specified, with up to 10 different cases used within a session. The intertrial interval (ITI) is designed to randomly vary between specified values about a specified mean. Different CSs and USs can be assigned to each of the 8 output bits controlled by the program. The duration of baseline sampling periods, the onset and duration of presentation of each stimulus, and the ISIs are all stored as part of a parameter file.

New experiment. This menu starts a new experiment after completion of a previous experiment or after a calibration 'Test Run'. This function clears data file names, trial numbers and data measures from previous sessions. Experiment control parameters are not cleared, to expedite continued training using the same paradigm.

Return to menu. Exits to the outer (utilities) shell.

2.1.2. F2: EDIT

These commands allow the user to edit the parameters controlling an experimental session, including the session (top left panel, Fig. 2), the trial cases (middle panel, Fig. 2), and the output bits assigned to each CS or US (top right panel, Fig. 2). At least 1 stimulus must be designated as a CS and 1 as a US for each session, even if only the CS is used (as in extinction studies) to allow standardized analyses of performance within designated intervals within the session (see the section on TBL files for a fuller explication). A 'dummy' US is entered solely for analytic purposes when the CS alone is actually presented to subjects.

2.1.3. F3: VIEW

Permits the user to examine stimulus controls or behaviors selected. Behaviors can be observed from both channels (as in Fig. 1) or from one single input channel, if desired (data from both are stored, if both are in use, irregardless of the display selected). The digital chart (Fig. 2, bottom panel) graphically displays the timing relationships, both logical and literal, used in each of the specified trial cases within an experimental session. The parameters edited with F2 can also be viewed but not changed with F3.

2.1.4. F4: RUN

Experimental sessions are initiated or re-started from this menu. Additionally, calibrating Test Runs can be performed, requiring no data file names (no data is stored to disk) but requiring use of a parameter file to control stimulus presentation. It is convenient to construct a parameter file with short ITIs for use in calibrating the interface hardware (refer to Thompson et al., 1994). Experimental sessions can be paused

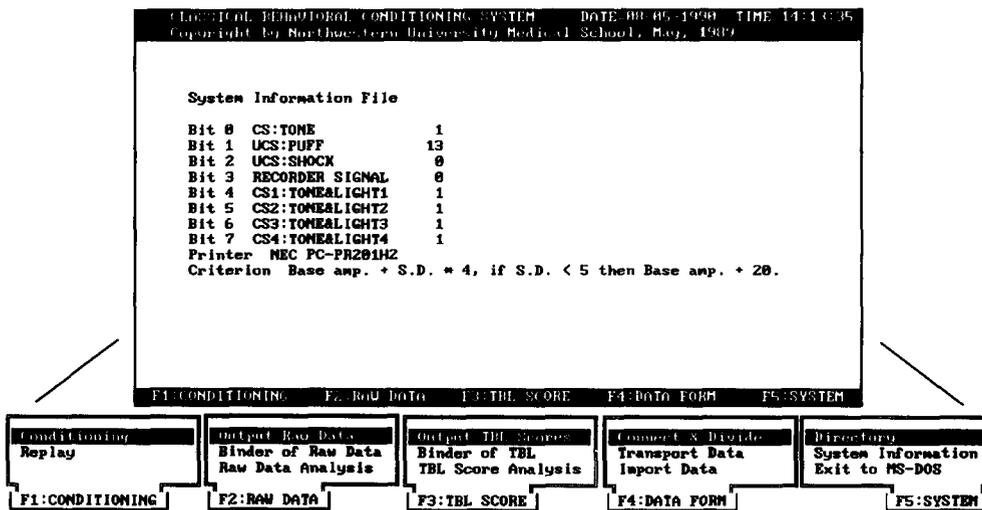


Fig. 3. The analytical and system utilities shell of NM500. Data can be replayed, printed, translated to other formats, averaged, combined, or sorted by stimulus condition with the utilities provided. DOS directories can also be viewed from within NM500. These integrated utilities eliminate the need for using multiple applications to manage quantitative data from associative learning studies. Multiple behavioral measures for statistical testing and presentation of results are generated (see Figs. 4 and 5).

using the Escape key (for example, to re-adjust eye-blink transduction) and re-started between trials without data loss. In the event of power failure or other inadvertent events, data is stored through the last trial actually run; additional trials can then be run as a new experiment and the 2 sets of data files produced merged with built-in utilities to produce a single experimental session.

2.2. Outer (utilities) shell

As illustrated in Fig. 3, the outer user interface shell allows access to NM500's file, display, and print utili-

ties and is logically separate from the main conditioning shell, which contains routines associated with control of conditioning sessions.

2.2.1. F1: CONDITIONING

This command allows the user 2 options.

Conditioning. Selects the inner experimental control shell (see Fig. 1) to set-up and run behavioral experiments.

Replay. This command allows off-line replay of an experimental session from disk, with a selected trial, a

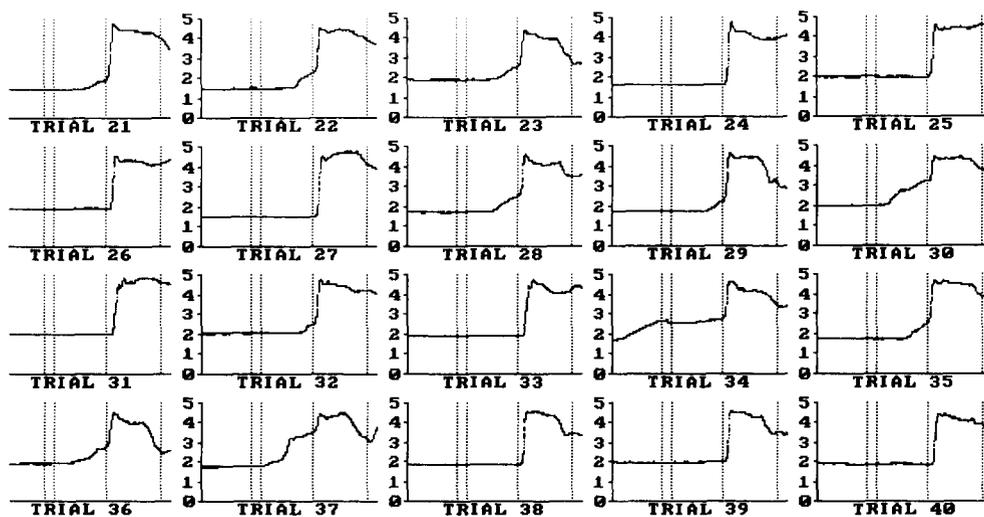


Fig. 4. A block of eyeblink response plots from 20 trials of a conditioning session. Response amplitude is plotted vertically, with time on the horizontal axis. No time scale is printed on these small plots (used primarily for archival purposes). Higher resolution plots with greater detail and scaling are also available as both printed output or in the form of graphics files that can be read by other computer systems.

series of trials, or the entire session displayed one screen at a time. This is useful for verifying the stability of behavioral performance measures, or for examining them closely in a more leisurely fashion than is possible under the time constraints imposed by most training paradigms. For example, in one study experimenters noted a preponderance of 'double-blink' responses within the brief ISI in trace-conditioned subjects receiving a glycine agonist (Thompson et al., 1992). This replay feature easily allowed these responses to be re-examined, counted, and accurately described.

2.2.2. F2: RAW DATA

This command allows output of individual or averaged eyeblink responses from an experimental session, as follows.

Output raw data. This utility outputs behavioral response graphs from one session to either a printer or to the display monitor. Fig. 4 is a sample of behavioral response graphs from twenty 500-ms trace-conditioning trials. The arrangement of the number of graphs tiled or viewed is adjustable in both horizontal and vertical axes to fit different paper and screen dimensions, and to allow for variation in the desired output resolution. One trial displays are possible (maximal resolution) as are displays of up to 9 × 9 trials (minimal resolution). When more than 1 screen is required to display the entire session, the up and down arrow keys increment or decrement trials. Behavioral graphs can be saved as image files for transfer to other programs.

Raw data analysis utility. This program calculates and displays averaged behavioral responses from an entire

session or from a range of trials. As in the previous utility, the user can print out or display these average graphs, or save them to an image file for export.

2.2.3. F3: TBL SCORE

Performance statistics are generated and stored for each training trial within an experimental session. These statistics can be reviewed, printed, or recomputed with the following utilities.

Output TBL scores. This utility outputs statistical tables (.TBL) from a session to a printer, to the display, or to a file on disk. The TBL file is in ASCII format, and can be opened by text editors or word processors. The header contains the user-defined statistical criteria that determined the onset of behavioral responses on-line. The 12 computed response variables described below are saved, 1 record for each trial within the session. In the print-out, these response variables are printed 1 line per trial, with the mean, standard deviation, and standard error for each for the entire session calculated and printed on the bottom line. Non-responses or non-CRs are shown as blanks in the output. Fig. 5 shows a sample .TBL file printout, with response variables calculated for each of the operationally defined intervals within trials. Recalculation of TBL analyses is possible, if the criteria for scoring responses are changed post hoc, if a.TBL file is lost, or if the intervals across which a response (e.g., the UR) is calculated must be changed.

2.2.4. F4: DATA FORMAT

Large data files can be split for convenient archiving on floppy disks, or can be re-assembled after a split

File Name : JT8681 DATE=11-29-1993 TIME=09:06:
 Criterion Base amp. + S.D. * 4, if S.D. < 5 then Base amp. + 20.

T#	Case	Cond.	ITI	Response		Base Line Period				Pre-UCS Period				UCS Period			
				R.onset	CR amp.	R. amp	Amp.	Peak Amp.	Peak Time	Amp.	Peak Amp.	Peak Time	Amp.	Peak Amp.	Peak Time		
1	1	CS-UCS	43	664.000		4145.613	1273.682	12.939	28.000	-11.424	3.174	18.000	2535.483	3546.875	720.000		
2	1	CS-UCS	59	664.000		4042.243	2754.250	26.511	85.000	-132.211	-18.655	14.000	1123.764	1887.517	731.000		
3	1	CS-UCS	55	326.000	2132.969	3082.131	1983.859	59.177	443.000	18.196	211.765	599.000	1452.873	1851.169	716.000		
4	1	CS-UCS	60	98.000	1836.348	3119.687	1983.491	13.813	268.000	-57.617	58.179	126.000	2486.208	2936.597	736.000		
5	1	CS-UCS	48	27.000	1671.283	2961.897	1938.828	44.402	485.000	-252.361	74.919	33.000	2366.830	2899.626	736.000		
6	1	CS-UCS	39	633.000		4522.679	1929.894	24.831	289.000	-145.173	-7.788	1.000	2426.186	2924.421	747.000		
7	1	CS-UCS	49	83.000	1557.799	2977.499	1628.118	17.398	481.000	-68.855	52.791	183.000	2683.898	3196.181	1088.000		
8	1	CS-UCS	48	637.000		4386.304	1737.327	14.382	128.000	-76.961	21.787	129.000	2385.948	3874.685	787.000		
9	1	CS-UCS	47	247.000	1694.243	3131.182	1651.233	21.138	5.000	22.486	65.876	573.000	2482.872	3165.662	781.000		
10	1	CS-UCS	46	643.000		4128.924	1547.881	18.281	94.000	-33.924	38.488	124.000	2375.386	3272.676	783.000		
11	1	CS-UCS	48	641.000		4178.948	1568.874	15.854	75.000	-68.862	2.847	8.000	2489.945	3254.888	699.000		
12	1	CS-UCS	53	658.000		4491.181	1982.988	254.648	188.000	-289.117	-51.748	1.000	2269.541	2848.898	737.000		
13	1	CS-UCS	50	648.000		4472.673	1623.183	11.418	388.000	-19.659	7.756	25.000	2598.658	3231.633	739.000		
14	1	CS-UCS	44	642.000		4468.816	1788.352	13.486	139.000	-163.163	8.523	13.000	2468.924	3852.957	748.000		
15	1	CS-UCS	48	629.000		4463.385	1716.614	15.564	359.000	-28.365	19.226	128.000	2598.645	3121.833	736.000		
16	1	CS-UCS	58	627.000		4575.452	1799.985	12.759	16.000	-44.895	13.979	22.000	2635.295	3851.889	736.000		
17	1	CS-UCS	47	628.000		4453.721	1782.329	15.288	16.000	-48.339	5.435	46.000	2687.248	3137.759	736.000		
18	1	CS-UCS	46	638.000		4168.166	1595.883	18.642	38.000	-48.178	8.281	6.000	2429.514	3221.891	687.000		
19	1	CS-UCS	48	627.000		4368.326	1781.726	15.883	83.000	-45.385	-2.587	3.000	2524.945	3153.818	752.000		

Fig. 5. An example of a tabular data (.TBL) file generated for an 80 trial experimental session. Trial number, case, and ITI are listed at left, while 4 sets of response measures are listed left to right. Performance is scored for each interval defined in the text, and is evaluated relative to the response amplitude measured within the baseline period. Blank cells in the CR amplitude column indicate trials on which no-conditioned responses were observed. Response onset latencies, mean amplitudes, and peak amplitudes are calculated, displayed on-line during conditioning, and saved to ASCII text files for internal use and for export to other programs.

with the connect and divide utility offered here. Data can also be converted from NM500's native file formats for export to other systems, or converted to ASCII string formats readable by NM500 using these utilities.

2.2.5. F5: SYSTEM

This set of utilities permits the user to view a DOS directory of files, to edit system wide hardware interface parameter information, or to exit to DOS from the NM500 shell.

File types. A number of file types are created by the NM500 system). Information files (.INF files) listing randomly generated ITIs and the trial types for all trials within a session are generated for each subject session and stored for use in later analyses. Stimuli are operationally defined as CS (up to 7 in number) or as an US, with 1 output bit on the A/D board assigned to each stimulus. Files indicating the assignment of stimulus output bits (SYSTEM.INF files) control data collection with differently configured hardware systems (e.g., with auditory CSs of different intensity or frequency, with tactile or visual CSs, or with alternate US channels). Stimulus timing parameters for use in different behavioral paradigms (.PAR files) can be re-used from session to session. These features save set-up time for training sessions and off-line analysis, and reduce operator errors during routine operation. Data files generated by NM500 and set-up files used in its operation are described below.

Table (.TBL) files. The results of numeric data analyses are called TBL measures, as the data are produced in the form of a table (see Fig. 5). Each eyeblink-conditioning trial is divided into 4 periods by the CS and US. The first period is called the baseline period, and lasts from the start of the trial until CS onset. The second or pre-US period lasts from the CS onset until US onset, the ISI. The third or US period lasts from the US-onset until US-offset. The fourth or post-US period lasts from US offset until the end of the trial.

All performance measures calculated by the NM500 software are based upon measures of the baseline behavior prior to presentation of the CS, or of the first CS, if more than one is used. The values read from the input ports of the A/D converter are treated separately for each subject and for each trial. Every amplitude calculated has the mean amplitude of the baseline period for that trial subtracted. The mean and standard deviation from that mean of all amplitude samples within the baseline period are automatically calculated for each trial. The experimenter predefines a statistical criteria for calculating responses differing from the baseline, and these criteria are stored in the SYSTEM.INF file, as are generally invariant for all experiments. Typically, an increase in the voltage output of

the eyeblink detector indicating a closing of the eyelid or nictitating membrane (see Thompson et al., 1994) is measured as a response when it exceeds a threshold more than 4 standard deviations above the mean baseline amplitude. In cases where the baseline standard deviation is very low (less than 5 mV), a voltage increment of 20 mV or greater is required for classification as an eyeblink response. Typical responses (see Fig. 5) are hundreds of millivolts to several volts in amplitude. A response occurring in the interval between CS onset and US onset is, by definition, a CR. A response occurring during US presentation is, by definition, an UR.

Twelve performance measures are calculated for each subject for each trial and saved as TBL files. The TBLs for each trial are divided into 4 groups. The first group of 3 measures includes response onset, response amplitude and conditioned response amplitude (if one occurs). The remaining 3 groups of TBL measures give the average amplitude, peak amplitude and the time of peak amplitude in each of 3 time intervals within the trial, the baseline period, the CS until US period, and the US period. Responses occurring in the post-US period are not analyzed.

The first group of TBL measures contains the onset latency of the first response that meets the predefined criteria as an eyeblink, and 2 amplitude measures related to that response. All times are calculated in milliseconds (relative to the trial start for baseline measures, or to CS onset for all other measures). All amplitudes are calculated in millivolts. Response onset is the time from CS onset to response onset. The response amplitude is the average amplitude from response onset to US offset (i.e., the average amplitude of both the CR and the UR, if both are present, or of the UR alone if no CR occurred). Conditioned response amplitude is the average amplitude from CR onset until US onset (this value is calculated only for trials in which a CR was detected). Note that these 3 performance measures have no value in some cases (see Fig. 5). If no response occurred prior to US offset, both response onset and response amplitude have no value. If a response occurred only during presentation of the US, response onset and response amplitude have values, but the CR amplitude does not.

The second group of TBL measures report the baseline amplitude. The first amplitude is simply the mean of all data sampled during the baseline period. As mentioned above, all other amplitudes are calculated after subtraction of this average baseline amplitude. Too high an average baseline amplitude can limit all other response amplitudes due to an artefactual floor effect. To avoid such problems, the program's parameter file limits the allowable baseline amplitude at the start of each trial. The program pauses and requests transducer adjustment to avoid this artifact. The peak

amplitude is the peak amplitude during the baseline period (i.e., the peak voltage above the average amplitude). This measure is useful for detecting intertrial responses (spontaneous blinks) that occur between the start of the trial and the onset of the CS. Peak amplitude time is the latency from the trial start until the peak voltage occurred. If 2 or more peaks of the same amplitude occur, only the latency of the first peak is listed in the table.

The next set of measures analyze behavior in the interval from CS onset until US onset (the pre-US period, during which conditioned responses are observed). Pre-US amplitude is the mean amplitude for the entire period. It correlates with but is not equal to CR amplitude, because it includes the average amplitude prior to the CR (or in the absence of the CR). Pre-US peak amplitude and pre-US peak amplitude time are useful for a detailed analysis of the CR. For example, the pre-US peak amplitude time detected temporal differences in CR latencies in hippocampectomized animals in both delay (Akase et al., 1989) and trace-conditioned (Moyer et al., 1990) rabbits.

The final set of measures analyze behavior during US presentation. US peak amplitude and US peak amplitude time are useful for a detailed analysis of the UR. Most responses continue after US offset, but an analysis of the UR during US presentation gives a good first-pass description of the response characteristics. If necessary, UR characteristics can be determined beyond the US interval in one of two ways. For example, recent work with human subjects (Disterhoft et al., 1992; Carillo et al., 1993) has demonstrated that aging subjects blink later in response to an airpuff than do young subjects. Analyses of blinks limited to the US interval alone would artefactually indicate that older subjects also exhibit smaller amplitude eyeblinks (since the amplitude of the response typically fails to peak until after the end of the US presentation), although examination of the complete eyeblinks indicates that amplitude differences between age groups are non-significant. The flexibility and extensibility of the NM500 software permits post hoc editing of the .INF files (see following section) to make it as if the US interval were much longer, allowing re-calculations of the performance of the full response. Since the files were re-analyzed after the actual airpuff presentations were a historical fact, stimulus timing relations were unaltered. Based upon these data, SYS.INF and trial case .PAR files were amended for training additional subjects, so that the actual airpuff was controlled by a formerly blank CS output bit, with identical timing relationships to the US previously used. While the US output bit actually controlled *no* stimuli, its *on* timing was lengthened to allow on-line analysis of the full UR by subjects of all ages. Thus, the NM500 system allows

for elegant modifications in its use midway through a study based upon outcomes arising from the data unforeseen at the start of a study.

Raw data information file (.INF). This file is an ASCII file that passes information from the conditioning program to the analysis program to reconstruct the actual data from conditioning trials. This file is saved when the conditioning session starts. An example of an information file follows:

```
NM500
U01203 01-13-1989 12:45:25
500 2000 1000 80 1 80 60 40 2
Case 1 0(0)-300(1)-400(0)-700(2)-850(0)
Case 2
Case 3
Case 4
Case 5
Case 6
Case 7
Case 8
Case 9
  1 1 1 46
  2 1 1 45
  3 1 1 43
  . . . . .
  . . . . .
79 1 1 45
80 1 1 60
```

The first line contains the version number of the software that collected this data set, as the NM500 analysis routines can analyze data gathered with earlier versions. The second line contains the name of the data file, and the date and time when the experiment was run. The third line contains information about the training session from the parameter file used. Included are the length of each data sample (in μ s); the number of samples per trial; the length of one trial (in ms); the number of trials per session; the number of trial types; the maximum, average, and minimum length of the ITI; and the baseline amplitude limit that stops a trial to avoid floor artefacts. The fourth to twelfth lines contain times (in ms) and output byte totals for trial cases from the parameter file. The analysis program decodes these lines into bit sequences, and determines the CS onset, CS offset, US onset, US offset and conditioning type (delay conditioning or trace conditioning) by reading the output bit assignment from the system information file (SYSTEM.INF). The thirteenth line onward contains information about each trial: the trial number, trial case number, trial type and ITI length. The trial type (CS and US = 1, US alone = 2, or CS alone = 3) is useful for examining responses from mixed stimulus paradigms.

The contents of the .INF file, the system informa-

tion file, and the .BEV file are sufficient for the analysis routines to reconstruct and analyze performances from a conditioning session. The user is not required to enter any values for these routines to function correctly, but only to select the desired .INF file from disk, minimizing errors during data analysis.

Raw behavioral data (.BEV). This is a binary file containing raw behavioral data (sampled by the A/D converter) at the specified sampling rate. Although each A/D data sample from the selected board is 12 bits, each sample is recorded by NM500 as 2 bytes (16 bits). Thus the file size is 2 bytes \times (number of samples/trial) \times (number of trials/session). The file is randomly accessed by analytic routines.

System information file (SYSTEM.INF). This is an ASCII file containing information about the assignment of individual bits on the digital output port, the system printer, and the statistical criteria used to define eyeblink responses. This file is read by most of NM500's subroutines, and controls conditioning and analytic functions. The information in this file usually remains constant, but can be edited by the set-up routines. When data collected with a different version of the SYSTEM.INF file is analyzed, the SYSTEM.INF file must be restored to the same state used for data collection to yield correct performance measures.

The following is an example of the contents of a SYSTEM.INF file:

```
Bit 0 CS:TONE           1
Bit 1 US:PUFF          13
Bit 2 US:SHOCK         0
Bit 3 RECORDER SIGNAL  0
Bit 4 CS1:TONEandLIGHT1 1
Bit 5 CS2:TONEandLIGHT2 1
Bit 6 CS3:TONEandLIGHT3 1
Bit 7 CS4:TONEandLIGHT4 1
Printer                NEC PC-PR201H2
Criterion Base amp.+ SD  $\times$  4, if SD < 5 then Base
amp.+ 20.
```

The first to seventh lines contain information about individual bit assignments for the digital output (see Fig. 2). The first letter of the bit name ('C', 'U', or other) determines whether the signal is the CS, US or other non-analyzed control signal. The delay time specified is used by the conditioning routines only, to adjust for physical timing differences required by specific output hardware (see Fig. 2, bottom panel). The eighth line contains information concerning the printer connected for output from the system. Many printers require different control codes for the graphic output from NM500. As currently written, the DEC LA100, several Toshiba printers, several NEC printers, and several Epson printers are supported. Alternately, image files can be saved and exported for printing on

other systems. The ninth line contains information about the response onset criteria used.

Parameter file (.PAR).* This is an ASCII file that details control of the conditioning sessions. Information from this file is copied into the .INF file during data collection, so that the analysis routines use this information to reconstruct the trials. The following is an example of a parameter file used for delay conditioning with a 400 ms tone CS, a 250 ms ISI and a co-terminating 150 ms puff US:

```
Length of 1 sample      500 ms
Number of samples in 1 trial 2000
Length of 1 trial       1000 ms
Number of trials        80
Number of trial cases   1
Length of ITI maximum  80 s
                      Average 60 s
                      Minimum 40 s
Limit amplitude to stop trial 2 V
```

Case 1 0(8)-300(1)-550(2)-700(8)

Case 2

Case 3

Case 4

Case 5

Case 6

Case 7

Case 8

Case 9

The first line is the duration of 1 data sample (in μ s). The second line is the number of samples per trial. The maximum permitted per trial is 4000. The third line is the length of 1 trial (in ms). This value is the product of the length of 1 sample and the number of samples per trial. The minimum length for 1 trial is 1 ms. The fourth line is the number of trials per session. The maximum number permitted is 200, the minimum 1. The fifth line is the number of trial cases used in conditioning. If this number is larger than 1 (as would be the case, for example, in discrimination training), the program randomly assigns each trial type to individual trials up to the total number of trials. The sixth, seventh and eighth lines contain information about the ITI: the maximum, average and minimum duration (in s). The conditioning routines randomly assign ITI values to each trial using these parameters. In pseudoconditioning, these ITIs are automatically cut in half, since the number of trials in each session is twice the number specified (CSs and USs are presented alone during pseudoconditioning, but the same number of times in the same total time as would elapse in a conditioning session). The ninth line is the baseline amplitude limit used to stop trials and avoid floor artefacts. The tenth to the eighteenth lines list trial cases. The value outside of parentheses specifies times (in ms) for the digital output, and the value inside of parentheses is

the byte data (sum of all output bits active or non-zero) appearing on the digital output from that time until the next time specified. The sequences are presented left to right, divided by a hyphen.

2.2.6. Other files

The NM500 system also creates other types of files for data analysis, conversion, or for transfer to other computer systems. These are briefly summarized below.

Information binder files (.IBT) and data binder files (*.DBT).* These binary files combine TBL information from multiple sessions for single subjects or for multiple subjects assigned to defined groups in factorial experimental designs. Data from these files are randomly accessed for plotting averaged behavioral performance curves for an entire session, for a range of trials within a session, for trials of a particular file type (e.g., CS alone or US alone trials) within a session, or for trials from more than 1 session.

Image files (.IMG).* An image file is a non-compressed binary screen dump, 640×322 pixels and 2576 bytes in size. It is possible to display and print out these screen dumps using the transform utilities. Also, these files can be opened as MacPaint™ files on Macintosh™ computers, using Macintosh utilities to change the file type to 'PNTG' and the creator code to one appropriate to the Macintosh graphics editor used (e.g., MacPaint™ = 'MPNT'; Canvas™ = 'DAD2'; Photoshop™ = '8BIM'; etc.). The image is rotated 90° to the left to fit the 576×720 pixel definition of MacPaint files. Image files make data from NM500 available for effective presentation without resorting to complex procedures to save and translate graphic images.

StatView files (.STA).* This is an ASCII table file containing the result of .TBL analyses, with data fields converted for use with other file formats, including LOTUS-123™ or other spreadsheet programs on the IBM-PC, or StatView™ and other statistical programs on the Apple Macintosh. We typically convert .TBL files into .STA files, save them on 3.5" high-density diskettes, and open them in StatView™ on a Macintosh with a floppy disk drive that reads both IBM and Apple formatted disks.

3. Results

3.1. System performance

One subject performing 2 tasks or 2 subjects each performing 1 task can be classically conditioned using

NM500. Virtually any combination of CS-US pairings, of experimental paradigms (e.g., trace, delay, or pseudoconditioning), or of stimuli may be used with the system. We have completed numerous experimental studies using delay (Akase et al., 1988) and trace conditioning (with 300 or 500 ms trace ISIs) (Deyo et al., 1989; De Jonge et al., 1990; Moyer et al., 1990; Thompson et al., 1992; Disterhoft et al., 1993a,b) with this software, both in its final form as reported here and in earlier developmental versions. Subjects have been trained in 50–160 trial sessions, but up to 200 trials per session are allowed for per data file. Training is easily stopped or re-started simply by pushing the appropriate function key. The on-screen display of the behavioral response in real-time allows the experimenter to monitor the subject without unwanted disruption (e.g., opening a training chamber door to verify eyeblink detector position). Overall system performance is further assessed by considering the accuracy of the system, its ease of use, and the frequency of system failures.

3.2. Accuracy

In a classical conditioning paradigm, the UR to presentation of the US is a reflexive or unlearned response. In eyeblink conditioning, the response is easily elicited by a corneal airpuff, and also occurs spontaneously. The baseline nictitating membrane activity is differentiated from CR or UR by the NM500 system. It uses a set of statistical criteria to define both CR and UR. When the amplitude of a blink occurring after CS onset is higher than the mean amplitude of the baseline period plus a multiple of the SD (typically the baseline amplitude plus 4 times the SD) of the baseline, the time of occurrence of this eyelid amplitude increment is defined as the response onset. This criterion takes into account small variations in eyeblink response amplitude which frequently occur during baseline periods. When the variation of baseline activity is extremely small, however, and the SD is close to 0, a very small amplitude increment could be artefactually defined as a response. To deal with these false positives, when the SD is less than 5 mV, the behavioral criterion is set as the average of the baseline period amplitude plus 20 mV. Experience with our system indicates that responses so defined are also visually identifiable in the raw data plots. Appropriate criterion can be set for other hardware systems, taking into account the gain of the eyeblink detector. In this system, the response onset criteria are not fixed but can be changed by updating the contents of the system information file (SYSTEM.INF) if the behavioral criteria selected prove inappropriate. The behavioral control routines refer to this system information file when defining response onset, and the replay module defines

the response according to the criteria defined in this file. Although response definition is done on-line during conditioning, off-line recalculation is also possible.

3.3. Ease of use

Since the NM500 is menu driven, all of the required commands are constantly available. The only instruction necessary is in the method of booting the system, use of MS-DOS for copying files, and functions of each command. The NM500 system is learned rapidly, usually in less than 1–2 h, and has been extensively used both in our laboratory and in others by a number of individuals with varying technical backgrounds with great success.

3.4. System failures

The NM500 system will crash when the hard-disk storage capacity has been reached. While the system can accommodate 200 data files (enough for 60 or more subjects with 80 trials per training session), there is no built in warning system which prevents the experimenter from starting a training session with insufficient disk space. In this case, the system will crash in the middle of an experiment. However, the partially collected data is not lost, since data is written to the hard-disk on a trial-by-trial basis. This problem can be readily averted by transferring the data to diskettes and removing these files from the system on a regular basis (weekly is suggested). A possible bug related to the random number seed generator occasionally causes the ITI to count up, rather than down. This bug causes no data loss, and is overcome by pausing and re-starting the trial with the Escape key and F4. No other known system failures have been encountered with NM500.

4. Discussion

The software described controls timing and delivery of CS, US, and other signals required for classical conditioning experiments, and records digitized behavioral response data on-line. The NM500 software analyzes the behavioral data both on-line, as each trial occurs, and off-line as a summary of trials within a session or of sessions within a complete training regime. Stimulus presentation and timing are controlled through digital outputs, with a parameter file delimiting specific conditions for each conditioning paradigm (e.g., delay, trace conditioning, pseudoconditioning, extinction). Conditioning paradigms may have one or several different types of trials in 1 training session. For example, during delay conditioning there is a simple presentation of a CS (e.g., a tone or light) which

precedes and overlaps the presentation of a US (e.g., airpuff or shock). Alternately, during pseudoconditioning there are trials in which only the CS is presented, and others in which only the US is presented, with the order of presentation randomized. Assignment of each digital output bit to a different CS, a US, or to another timing signal (e.g., for marking trial onset on a taped record of the trial) is permitted. The NM500 system has been written to adjust for response lag times in the mechanical operation of experimental equipment. For example, the arrival of the airpuff at the cornea may be delayed many milliseconds after the electronic signal orders opening of a solenoid valve to deliver the pressurized air. Adjustments for such mechanical hysteresis can be easily made by the experimenter.

In summary, we have developed a software system designed to control all of the necessary experimental events and to collect, store, and analyze all data required for classical associative learning studies, including studies of eyeblink or nictitating membrane responses¹. The system is designed to be used on common and relatively inexpensive microcomputers (80286-based IBM PC-AT compatibles) that are already available in many labs, and need not be dedicated solely to experimental use. The software provides excellent graphic displays of behavioral responses in real-time, allowing the experimenter to make necessary adjustments in the positioning of the eyeblink detector, bypassing the need for an oscilloscope. Numerous experimental paradigms can be used for training within a given day's experiments without requiring modification of the hardware and without changing the software code. The system provides a useful tool for reliable acquisition and quantitative analysis of classically conditioned responses.

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References

- Akase, E., Alkon, D.L. and Disterhoft, J.F. (1989) Hippocampal lesions impair memory of short-delay conditioned eyeblink in rabbits. *Behav. Neurosci.*, 103: 935–943.
- Carrillo, M.C., Thompson, L.T., Naughton, B.J., Gabrielli, J., and Disterhoft, J.F. (1993) Aging impairs trace eyeblink conditioning in humans independent of changes in the unconditioned response. *Soc. Neurosci. Abst.*, 19: 386.
- De Jonge, M.C., Black, J., Deyo, R.A. and Disterhoft, J.F. (1990)

- Learning-induced afterhyperpolarization reductions in hippocampus are specific for cell type and potassium conductance. *Exp. Brain Res.*, 80: 456–462.
- Deyo, R.A., Straube, K. and Disterhoft, J.F. (1989) Nimodipine facilitates trace conditioning of the eyeblink response in aging rabbits. *Science*, 243: 809–811.
- Disterhoft, J.F., Conroy, S.W., Thompson, L.T., Naughton, B.J., and Gabrielli, J.D.E. (1991) Age affects eyeblink conditioning and response discrimination in humans. *Soc. Neurosci. Abst.*, 17: 476.
- Disterhoft, J.F., Moyer, J.R., Jr., Thompson, L.T. and Kowalska, M. (1993a) Functional aspects of calcium-channel modulation. *Clin. Neuropharmacol.*, 16: S12–S24.
- Disterhoft, J.F., Thompson, L.T., Moyer, J.R., Jr. and Kowalska, M. (1993b) Nimodipine facilitates learning and increases excitability of hippocampal neurons in aging rabbits. *Drugs Develop.*, 2: 395–405.
- Lavond, D.G. and Steinmetz, J.E. (1989) An inexpensive interface for the IBM PC/XT and compatibles. *Behav. Res. Methods, Inst. Comp.*, 21: 435–440.
- Moyer, J.R., Jr., Deyo, R.A. and Disterhoft, J.F. (1990) Hippocampectomy disrupts trace eye-blink conditioning in rabbits. *Behav. Neurosci.*, 104: 243–252.
- Scandrett, J. and Gormezano, I. (1980) Microprocessor control and A/D data acquisition in classical conditioning. *Behav. Res. Methods Instru.*, 12: 120–125.
- Solomon, P.R. and Babcock, B.A. (1979) KIM and the rabbit: the use of the KIM-1 microprocessor to control classical conditioning of the rabbit's nictitating membrane response. *Behav. Res. Methods Instru.*, 11: 67–70.
- Thompson, L.T., Akase, E., Moyer, J.R., Jr. and Disterhoft, J.F. (1994-companion paper) A system for quantitative analysis of associative learning. 1. Precision stimulus presentation and response transduction. *J. Neurosci. Methods*, 54 (1994) 109–117.
- Thompson, L.T., Moskal, J. and Disterhoft, J.F. (1992) Hippocampus-dependent learning facilitated by a monoclonal antibody or D-cycloserine. *Nature*, 359: 638–641.