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A system for quantitative analysis of associative learning. Part 1. Hardware interfaces with cross-species applications

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Abstract

This paper describes a reliable, durable, and readily calibrated hardware interface system designed to present sensory stimuli at precise time intervals and to transduce and digitize behavioral data in classical conditioning experiments. It has been extensively tested in a 'model'-associative learning task, conditioning of eyeblink or nictitating membrane responses, but is readily adapted to other behavioral paradigms. Each system can run a pair of conditioned experimental or pseudoconditioned control subjects simultaneously, or collect data from a single subject carrying out two tasks simultaneously. The requirements of the system are defined, based around an inexpensive AT-class MS-DOS microcomputer. The interface hardware needed to present auditory tone conditioned stimuli and corneal airpuff-unconditioned stimuli to training subjects are detailed, with timing signals provided by TTL pulses generated at the digital output ports of an analog-to-digital (A/D) converter. An electronic circuit is described that provides stable inputs to the A/D converter, transducing eyeblink responses to voltage signals opto-electronically, without requiring any invasive attachment of the subject to the measuring device. The 1-piece eyeblink sensor used (selected for ease of alignment and maintenance) is also discussed. Examples of applications for classical conditioning of rabbits, rats, and human subjects are described. A companion paper describes data-acquisition and control software written as a user-friendly interface for this hardware system.

Keywords: Eyeblink conditioning; Nictitating membrane response; Associative learning; Solid-state circuitry; Opto-electronic sensor; Digital signal transduction

1. Introduction

Many research groups have adopted classical conditioning paradigms as 'model systems' to analyze the neurobiological substrates of associative learning in vertebrates (Disterhoft et al., 1977; Thompson, 1986; Woody, 1986) and invertebrates (Carew and Sahley, 1986; Kandel et al., 1986; Mpitstos and Cohan, 1986; Alkon et al., 1987). Classical conditioning of eyeblink responses was first used to study human learning (Gormezano and Moore, 1962) and was later adapted by Gormezano et al. (1962), using the albino rabbit, to provide an animal model of a non-verbal learning task. In classical conditioning/Pavlovian conditioning/associative-learning paradigms (the terms are often used interchangeably), an initially neutral stimulus (the conditioned stimulus (CS), e.g. a brief 6 kHz tone) which fails to elicit a reflexive or unconditioned response (UR) is 'paired with' (presented prior to the onset of) an unconditioned stimulus (US, e.g., the aroma of food) which elicits a reflexive UR (e.g. salivation). After a number of trial pairings, the formerly neutral CS appears to elicit behavioral responses associated with the US, and eventually elicits a conditioned response (CR) in the absence of the US.

There are several advantages of classical conditioning paradigms for neurobiological analyses of learning. Most notably, the experimenter, rather than the test subject, has rigorous control over stimulus presentation and trial timing. Because there is a defined temporal

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sequence from CS presentation to CR output, systematic studies of neural systems involved in the conditioned and unconditioned response pathway have been possible (see Disterhoft et al., 1987). Classical conditioning studies of eyelid or nictitating membrane blink responses have been particularly popular, due to the relative ease of eliciting the response in a number of species, including humans (Gormezano and Moore, 1962), rabbits (Schneiderman et al., 1962), cats (Woody and Brozek, 1969), rats (Skelton, 1988), and chickens (Davis and Coates, 1978); to a lack of painful or injurious stimulation of subjects when training uses tone CSs and mild corneal airpuff USs; and to numerous ingenuous methods developed during the past 3 decades for transducing the response. The eyeblink response itself is discrete, requiring no gross motor movement or ambulation on the part of the subject, which greatly facilitates neurophysiological studies of the underlying neuronal substrates, including extracellular or intracellular recording of single-neuron activity (Berger et al., 1976; Kraus and Disterhoft, 1982; Woody et al., 1991) or PET studies of local cerebral blood flow (Zeffiro et al., 1993) during training. Variations in sensorimotor responsiveness to the US, in the form of altered amplitude, latency, or even absence of the UR, are measurable throughout training. Good control procedures have been described and widely used, including pseudoconditioning (using explicitly unpaired presentations of the CS and the US) (Disterhoft et al., 1986; Deyo et al., 1989) and extinction (CS alone presentations after acquisition occurs) (Akase et al., 1989; Thompson et al., 1992). Higher order variants of these paradigms have also been used, including trace conditioning (Solomon et al., 1986; Moyer et al., 1990), blocking (Kehoe et al., 1981), overshadowing (Kehoe, 1982), stimulus discrimination (Frey and Ross, 1967), pairing with very long or very short interstimulus intervals (Frey and Ross, 1968), and discrimination reversal training (Berger and Orr, 1983).

This paper and its companion (Akase et al., 1994) describe what we have found to be reliable methods for eliciting, training, and quantifying eyeblink or nictitating membrane responses. The present paper describes in detail the necessary solid-state electronic equipment required for transduction of blink responses, using readily available components. The eyeblink detector is integrated with other components of an experimental control system, controlled by a software system described in the following paper. The complete system offers precise control of stimulus presentation coupled with ease of analysis for eyeblink conditioning and other associative learning paradigms. Our methods require no physical attachment of the face or eyelid of the test subject to the measurement device. The methods described have been successfully used for a wide variety of studies, ranging from straightforward conditioning or pseudoconditioning of rabbits for later use in in vitro biophysical studies (Moyer et al., 1993; Thompson et al., 1993); neurobiological studies of the effects of lesions (Akase et al., 1989; Moyer et al., 1990) or pharmacological studies of the effects of drugs (Deyo et al., 1989; Thompson et al., 1992) on acquisition, retention, or extinction; and analyses in vivo of hippocampal neuronal plasticity during acquisition of eyeblink conditioning (Akase et al., 1988; Weiss et al., 1993). The combination of hardware and software detailed has been used for eyeblink conditioning of rabbits, rats, and humans, and yields data that is useful for theoretical comparisons between animal model and human systems of learning.

2. Hardware interface overview

An IBM-AT-compatible (Intel 80 × 86-based) microcomputer with at least 640 kbytes of DRAM, a 40 Mbyte or larger hard disk, an EGA or better video card, a 640 × 480 pixel or higher resolution color monitor is required. We have used '286, '386, and '486 machines, with a range of clock speeds from 20 to 50 MHz, from a wide variety of PC clone manufacturers with equal success (the A/D board used has its own internal timing oscillators that ensure consistent performance across different platforms). The machine used must provide at least one fully bussed AT-compatible backplane slot for the A/D converter board described below (the card is half-sized, increasing compatability with even small footprint computer designs). Additional RAM installed in the PC can be used for terminate-and-stay-resident applications (TSRs) without interfering with the system's operations. The microcomputer can also be used for other applications besides classical conditioning (e.g., word processing, statistics, telecommunications, etc.) between experimental sessions. At least a 10 Mbyte partition on the hard disk should be reserved for data collection, however, to allow for storage of the multiple types of data files generated by the experimental control software (see Akase et al., 1994). In practice, daily backups and removal of data files from the hard disk are advisable. An A/D converter board (DT2811, Data Translation, Marlboro, MA) is a required central component of the hardware (and software) system. This board provides 3 subsystems, 2 of which (the A/D and digital I/O subsystems) are used by the software described in Akase et al. (1994). The 20 kHz 12 bit A/D subsystem supports up to 16 analog inputs (the software supports up to 2) in the range 0 to + 5 VDC (a maximum input voltage of ±32 VDC is possible without damage), with minimal crosstalk and very low error rates. The TTL control signals for the stimulus hardware described below are provided by the digital I/O subsystem of the

board, which has two 8-line ports (8 input and 8 output bits, with only the output bits supported by the software). The bits provide nominal TTL pulses on the order of 2.4 V at 15 mA, more than sufficient to drive the hardware described.

A block diagram of the hardware components of a basic classical conditioning system are shown in Fig. 1. For simplicity, 1 CS, 1 US, and 1 input pathway are detailed. As described below, some components of the CS pathway can be replicated to allow for use in more complex behavioral paradigms such as discrimination learning, or substitutes can be used if non-auditory stimuli are needed (e.g., DC-powered lamp can provide a visual CS). Identical sets of eyeblink detector inputs are normally used to condition a pair of subjects simultaneously. For other studies, an eyeblink detector and an alternate input device have been used to measure performance of 1 subject on 2 behaviors simultaneously (a simple reaction-time measurement is described below). Although it is possible to build all elements of the system from discrete components (several physically disimilar but functionally equivalent sets of hardware were used for several years in our laboratory, with the differences dependent upon component price and availability at the time of assembly), we prefer whenever possible to standardize the logic control circuitry using commercially produced equipment (see Table 1 for examples). Issues related to ease of use and maintenance, ease and timeliness of replacement, portability, and durability are simplified by use of commercial equipment. The microcomputer and the associated hardware can be mounted on a standard laboratory rack system, or can be placed on a bench or tabletop for convenient access. A grounded AC power source is required. The airpuff stimuli can be supplied by a well-regulated and filtered air compressor (often connected to built-in pipes in research environments) or by use of securely mounted tanks of compressed air or nitrogen. Proper regulators suitable for the gas selected are required for safety.

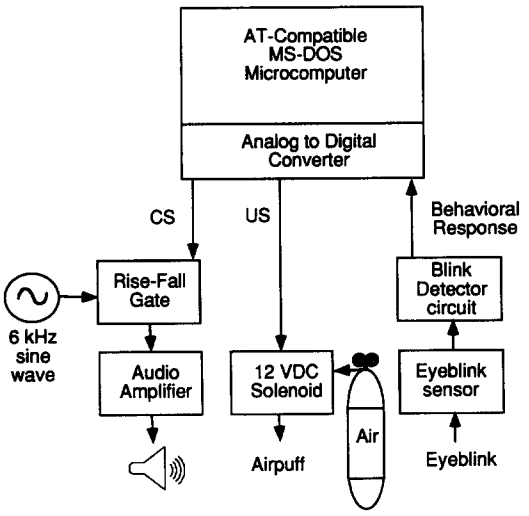

Fig. 1. Block diagram of the hardware system for controlling stimulus presentation and for transducing behavioral data for on-line analysis in associative eyeblink conditioning experiments. The hardware interfaces with a commercially available PC-AT class microcomputer via a 16-bit A/D converter installed in 1 of the PC's bus slots. In simple applications, 2 output bits control timing of external hardware gating a tone signal (CS) and an airpuff (US). Up to 8 outputs are available for more complex stimulus control. The eyeblink or nictitating membrane response is transduced into an electrical signal via an Optek OPB704, that combines a LED and a phototransistor in a single, prealigned package for easy set-up and maintenance. Use of the blink sensor is described fully in Fig. 2.

Table 1

The following modules (Coulbourn Instruments, Allentown, PA) readily assemble in a self-contained rack mounted frame with ±12 VDC power supply and the necessary interconnections to provide an easily maintained logic control system for the hardware interface described (equivalent products are available from other manufacturers, or can be custom built in the laboratory as needed). Several additional modules beyond those described in Fig. 1 are used. One converts standard TTL logic pulses supplied by the microcomputer's A/D board into the −12 VDC logic used by Coulbourn [1]. One provides a +5 VDC reference [2] for detection of TTL pulses by the gate-controlling CS presentation. One boosts the power of the signal gating the US [3] to a level sufficient to operate the solenoid regulating airpuff delivery. A set of switches [4] allows manual testing and calibration of both CS and US (recommended at least once daily prior to use; more frequently if multiple settings are used). A set of indicator lights [5] conveniently indicate presentation of various stimuli to subjects tested in enclosed, soundproofed chambers.

[1] S22-06 5V logic to 12V logic converter
[2] S15-01 5V power supply
S84-04 shaped rise/fall gate
S81-06 precision signal generator
S82-24 audio-mixer-amplifier
[3] S61-05 power driver
[4] S96-24 quad buffered switch
[5] R10-10 LED indicator

A simple well-labeled interface panel can be used to 'fan out' 2 input and 8 output bits of the A/D board, allowing direct connection of the equipment used for stimulus control and behavioral measurement. Blank panels of thin sheet aluminum or steel are used in our laboratory, with appropriate holes drilled for installation of BNC bulkhead connectors, assuring reliable electrical interconnections. Switches should be installed to allow routine manual testing and calibration of CS and US stimulus intensities. These switches temporarily disconnect individual output bits from the A/D board, substituting continuously high external TTL logic level signals (+5 VDC) to the connectors driving the external logic components. The major elements of the hardware interface are detailed below, beginning with the analog inputs to the A/D board.

3. Eyeblink detector

The input side of the hardware, which transduces eyeblink responses using reflected light and custom solid-state circuitry, is described below. Other methods have relied upon physical attachment of the eyelid or nictitating membrane (via a suture) to a resistive or mechanical motion transducer, or upon use of electrodes around the orbit to convert muscle activity to an EMG signal. The advantages of our non-invasive methods include stability of operation, ease of use, and the comfort advantages inherent in a system which does not require a direct physical connection of the eyes or face of human or animal subjects to a transduction device or sensor. The blink detection apparatus consists of 2 parts, a 1-piece opto-electronic sensor device for converting the actual eyeblink into an electrical signal, and the blink detector circuitry. The 2 parts are connected to one another with shielded 4-conductor cable (in applications where electrophysiological recording is not required, unshielded modular telephone cable can be used).

3.1. Eyeblink sensor

The opto-electronic sensor used for transduction of the behavioral response is an Optek OPB704 (TRW Electronics, Carrollton, TX; similar devices, some using infra-red emission, are available from other manufacturers). The Optek OPB704 actually contains 2 discrete devices (a light-emitting diode (LED) and a phototransistor) packaged together with focusing lenses in a rigid acrylic shell. The LED emits a focused beam of low-intensity infrared light that is reflected back from the corneal surface, with a focal point 4 mm from the sensor. The amount of reflected light is converted to a DC voltage signal by the phototransistor. The optimal angle of reflectance is built in, so that the device provides a maximal voltage output when a reflective surface crosses the focal point, and a null voltage when a transparent surface occupies the focal point. The translucent surface of the cornea reflects a smaller amount of the light emitted by the LED than the opaque eyelid or nictitating membrane. The phototransistor output converts this variation in light reflectance into a range of voltage outputs analogous to the eyeblink, which is then amplified suitably by the detector circuitry, providing a 0–5 VDC range of inputs for discrimination by the microcomputer software (see Akase et al., 1994).

The 1-piece sensor package selected has proven much easier to maintain than 2-piece LED/phototransistor arrangements used extensively in past behavioral studies in our laboratory (Disterhoft et al., 1977; Akase et al., 1989; de Jonge et al., 1990; Moyer et al., 1990). The angle between the LED and the phototransistor is preset and fixed, preventing alignment induced artefactual errors. The small plastic lenses covering the front of the sensor make it relatively impervious to damage from fluids, including ocular secretions. Cleaning of the lenses is easily accomplished using cotton swabs, ethanol, and distilled water. The Optek device can be connected to a PC-mount phone jack (SPC Technologies, TA-258-4) for cable interconnection, and has a central screw slot that can be used to mount the sensor to an external holder for experimental use. Fig. 2 details a holder, which allows optimal alignment of the sensor to the corneal surface relative to the geometry

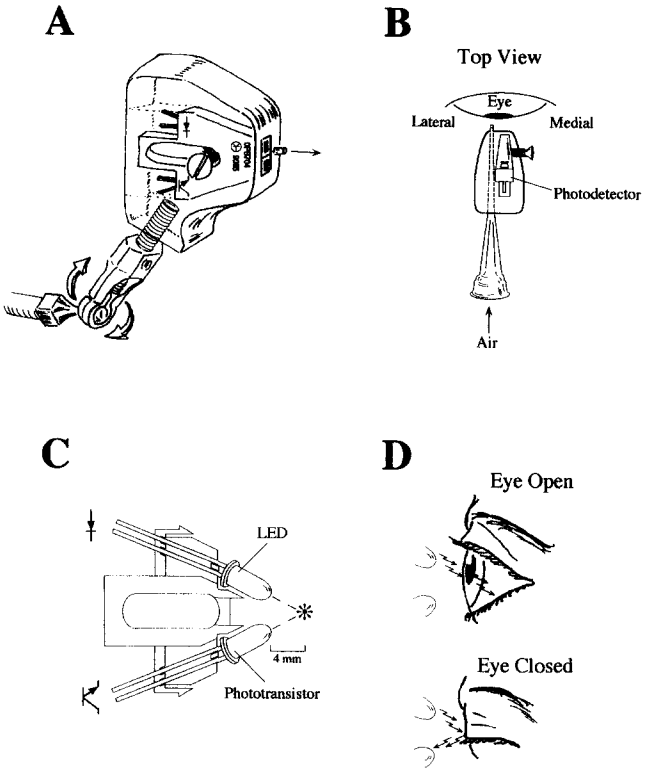

Fig. 2. Components of the non-invasive eyeblink detector. A: Optek sensor is shown within a machined piece of Plexiglas, supported on a threaded rod that pivots via a ball joint attached to another rod. It can then be supported by a clamp on a magnetic base (for animal studies) or attached to a headband or hat (for human studies). The swivel permits placement of the detector to maintain proper alignment and distance from the subject's eye. B: top view showing relative positions of the airpuff pipette and the detector within the holder. The detector is directed at the center of the eye (treated as if it were a perfect sphere) to optimize the physics of reflectance, while the pipette delivers the airpuff to the lateral surface of the cornea. The Optek sensor is secured within its holder by a nylon screw. C: within the Optek OPB704, the LED and phototransistor are assembled at a fixed angle, yielding a fixed optimal reflectance distance ('sweet spot' *). D: when the detector is placed near the open eye, very little light is directly reflected back (most scatters within the eye). When the subject blinks, closing the eyelid or nictitating membrane, much more light is reflected from the relatively opaque eyelid back to the phototransistor. The resulting change in output voltage from the phototransistor is amplified by the blink detector circuit (see Fig. 3) for subsequent digitization.

of the eyelid or nictitating membrane, and standardizes airpuff stimulation. For human applications, the holder is used attached to a headband with Velcro adjustments that permit the sensor to be placed in proper alignment to the subject's eye. In use, the lenses of the Optek device are aligned normal to a plane through the retina of the eye, with the LED above and the phototransistor below, directly facing and approximately 4 mm from the center of iris. A pipette tip attached via latex supply tubing to the airpuff solenoid valve is mounted parallel to the lenses, offset 3 mm from the side of the sensor, so that the airpuff strikes the caudal or lateral portion of the cornea (dependent upon the species tested). The unconditioned nictitating membrane response (NMR) of a rabbit consists of a rostral to caudal extension of the nictitating membrane. As the nictitating membrane moves past the reflecting point of the sensor, the opaque membrane reflects back a greater amount of light into the phototransistor, yielding a higher voltage output from the blink detector circuit. The dorsal to ventral closure of the rat or human eyelid similarly alters the light reflected back to the phototransistor. The physical alignment of the sensor to the eye as shown in Fig. 2 is critical to accurate transduction of the behavioral response.

3.2. Blink detector circuit

The blink detector circuit is based around an inexpensive operational amplifier (Texas Instruments TL072; a complete parts list is included in Table 2). Fig. 3 shows the schematic of the blink detector circuit. An external power source supplying ±12 VDC is required, and also powers the airpuff solenoid and other interface hardware. The circuit consists of a rectified power supply section, giving the LED and the phototransistor the appropriate supply voltages (the operational amplifier chip is supplied with + 12 VDC and − 12 VDC on pins 8 and 4, respectively), an amplifier section with appropriate biasing resistors to amplify the output of the phototransistor, and an output noise filter. The output voltage is buffered so that it can be fed directly to the computer system via the A/D board, to an oscilloscope or voltmeter, or recorded on an analog or digital recording system. This circuit provides stable output both in total darkness and in the subdued ambient light conditions found in our laboratories; in fact, either fluorescent (40 W tubes) and incandescent (40 W bulb) lights illuminating the training room have no significant effect on the circuit's output in daily use, provided they do not directly reflect off the subject's cornea into the phototransistor (direct photic input to the sensor in normal use is precluded by proper alignment with the eye, as shown in Fig. 2). Maximal output from the circuit is adjusted to + 5 VDC using the variable resistor R5 for each set of eyeblink sensors used. In our experience, this adjustment exhibits little or no drift over more than 1 year's daily use of the same equipment, although more frequent calibrations are recommended.

Table 2
Parts list for 1 complete blink detector circuit. Two complete circuits with all input and output connections fit on a single 4×6″ PC board.

Part	Value
Capacitors	
C1, C2	47 μF, 25 VDC electrolytic
C3	10 μF tantalum
C4	2.2 μF tantalum
Resistors (all 5% carbon film)	
R1, R4	1.2 kΩ
R2	4.7 kΩ
R3	220 kΩ
R5	4.7 kΩ PC mount potentiometer
R6	33 kΩ
Diodes	
D1	1N914
Sensors (packaged LED/phototransistor)	
Optek OPB704	
Integrated circuits	
TL072 dual operational amplifier	
Additional parts	
Banana jacks	
BNC jacks	
Modular telephone plugs and jacks	
8 pin IC socket	

We have found it convenient to construct the detector circuits in pairs on a single board, yielding a complete set-up for running paired control and experimental animals simultaneously. We first etch the interconnections on double-sided copper clad PC boards (Kepro Circuit Systems, Fenton MO; P2-465-B). This eliminates the need for hand wiring numerous components, and allows relative novices to completely assemble the

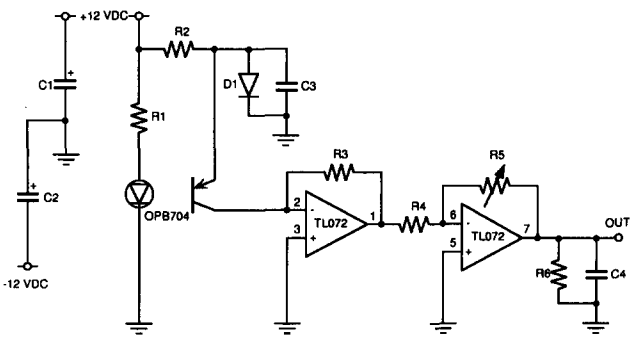

Fig. 3. Schematic of the blink detector circuit. Using a TL072 dual operational amplifier, the circuit amplifies, buffers and filters the voltage output of the Optek OPB704 sensor. Output from the blink detector circuitry is suitable for display on a standard oscilloscope and/or for digitization (via the A/D board) on the microcomputer for analysis. A complete parts list is shown in Table 1. Typically, 2 complete detector circuits are laid out, etched, and mounted on a single 4×6″ double-sided copper clad printed circuit board.

boards. The single integrated circuit is socketed, for easy maintenance. All other components are soldered to the boards. Modular phone jacks (4-conductor) are used for connection to the Optek sensor, female BNC connectors provide output connections to the A/D board, and female banana connectors supply the power and ground. The detector circuit board is mounted outside the conditioning chamber used for behavioral training, while the 1-piece sensor is placed inside the chamber near the eye (see Fig. 2).

3.3. Alternate input devices

This hardware interface is designed to accept inputs from devices other than the blink detector circuitry detailed above. For example, we have used this system to study differential reaction times to different CSs in human populations. A simple trigger device was connected to input port 2 via a simple resetting 1-shot circuit that emitted a 120 ms duration 5 V square-wave pulse each time the trigger was pulled. Other devices generating a voltage signal in the range of ± 10 VDC (to avoid exceeding the specified input levels for the A/D converter selected) can also be used. The software used (see Akase et al., 1994) is currently configured to analyze inputs in the range from 0 to + 5 VDC. Use of inputs outside this voltage range would require changing jumpers on the A/D converter board.

4. Implementing the system for behavioral studies

4.1. Conditioned stimulus presentation

In our current experiments, the CS presented to subjects is a 100 ms duration 6 kHz pure tone, delivered binaurally via stereo headphones. CS duration is controlled by the experimenter via the conditioning software (see Akase et al., 1994). The tone is generated by a sine wave generator. The sine wave is gated by 1 of 8 TTL output bits (software selected) from the A/D board, using a shaped rise/fall gate. A signal is present on the output of the gate only when the TTL gating signal is positive. The tone signal is amplified using a high-fidelity audio-amplifier and output via standard audiophile quality headphones or speakers. Headphones have proven preferable, since with the addition of latex tubing to the earpieces they are adaptable to replicable placement and use in non-human species. This arrangement yields an audio-signal whose onset and offset characteristics, as well as pitch and duration, are invariant between presentations. The temporal characteristics of the CS tone can readily be tested by placing a microphone adjacent to the speakers or earpieces, amplifying the microphone output suitably, and examining the audio-signal on an oscilloscope triggered

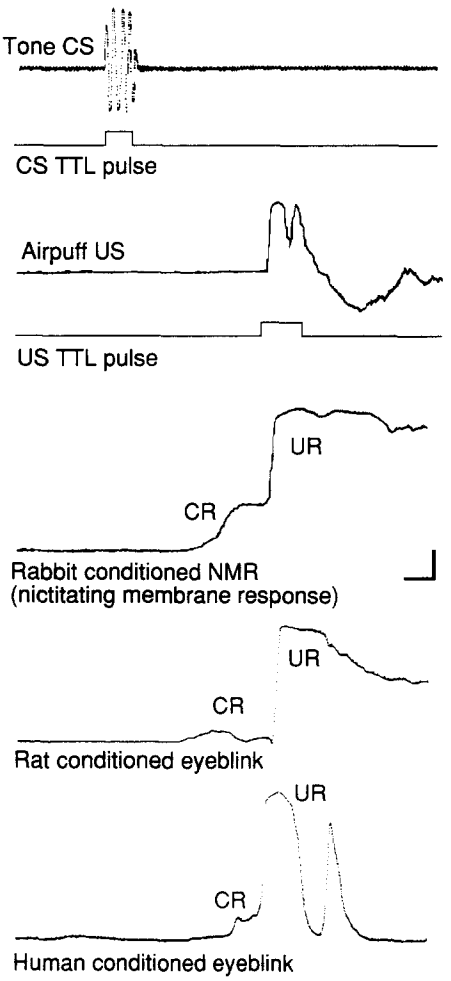

Fig. 4. Digitized display of the relation of timing signals for a tone CS and airpuff US and for conditioned behaviors measured as the output of the blink detector circuit. This figure illustrates timing relationships for subjects from 3 species trained in the 500-ms trace eyeblink conditioning paradigm. To produce a 500-ms interstimulus trace interval, the digital timing signals have been adjusted to allow for mechanical hysteresis in the airpuff solenoid and impedance of the supply tubing delivering the US (note the time lag between the onset of the US TTL pulse and onset of the US itself). The subjects reliably emit CRs in the trace interval that are predicted by the CS, precede onset of the US, and overlap URs elicited by the airpuff US. This non-invasive behavioral transduction system yields very stable responses across sessions and across groups for all species tested.

by the TTL pulse from output bit no. 0 (see Fig. 4). In use, the time lag between the rising edge of the TTL pulse and tone onset is less than 1 ms, and tone duration varies by less than 1%. Calibration of the sound intensity is readily accomplished using a hand-held sound-level meter (Radio Shack, Tandy 33-2050, or General Radio, 1565-B).

4.2. Unconditioned stimulus presentation

The airpuff US used is a 150 ms duration jet of room temperature gas (both nitrogen or compressed

air have been used, with equivalent results). The airpuff is vented through a plastic microliter pipet tip, placed approximately 2 mm from the cornea of the eye to be conditioned, and directed at the caudal or lateral corner of the cornea (see Fig. 2). The US duration is controlled by the microcomputer software (Akase et al., 1994), while US intensity (typically 3.0 psi) is mechanically controlled by a 2-stage regulator connected to the air source (Harris Calorific, Chicago, IL, Model 92SS-15). The airpuff is delivered using a 12 VDC solenoid valve (Atkomatic Valve, Indianapolis, IN, no. S-2406-VN) via latex tubing to the pipette tip placed near the cornea. Again, a microphone should be used to measure the mechanical timing properties of each solenoid used at the desired output air pressure, as individual solenoids and different lengths of supply tubing produce varying lag times (ranging between 3 and 15 ms, in our experience) between TTL pulse onset and actual airpuff onset. The microcomputer software (Akase et al., 1994) is specifically designed to allow for adjustments in the timing of TTL signals to compensate for differences in the physical response characteristics of actual output components. Extremely precise control of the onset and duration of both the CS and the US is thus achieved.

Additional CSs or USs can be delivered using additional A/D bits, up to a total limit of 8 stimuli with the A/D board selected. For example, in current human applications (see Disterhoft et al., 1991; Carillo et al., 1993) additional CS outputs were used to control 2 different light CSs, for use in concurrent stimulus discrimination testing. Complete temporal control of the presentation of defined sensory stimuli is possible, compensating for the mechanical or physical properties of the devices used. Stimuli can be presented consecutively, concurrently, overlapping in time, or in varying combinations across individual trials. Thus, a variety of experimental paradigms can be directly controlled using this system.

4.3. Behavioral measurement

As an example, for many of our experiments rabbits are trained in pairs in separate darkened, sound-attenuated chambers. Rabbits are restrained using cloth snug bags with drawstrings front and rear, to prevent movement-induced injuries. They are also placed in padded Plexiglas stocks similar to those described by Gormezano et al. (1962). Rabbits are habituated to restraint and to the experimental chambers for at least a 1-h session in the week before training begins. Rabbits tolerate restraint and presentation of the conditioning stimuli remarkably well — a major reason this behavioral preparation has been used extensively in our laboratory for many years (see Disterhoft et al., 1977). The eyelids are held open with small eyeclips attached to Velcro straps for easy adjustment, so that nictitating membrane (third eyelid) extension can be measured independent of eyelid responses. The sensor is mounted parallel to the bottom of the conditioning chamber on an adjustable magnetic base. In our work with other animal species lacking a nictitating membrane, no eyeclips are required and eyeblink responses are measured. For human subjects, verbal instructions are given to sit quietly (often supplemented with presentation of a video or other amusement). Fig. 4 illustrates typical stimulus-response relationships for tone CSs and airpuff USs, with behavioral data shown for conditioned eyeblinks performed by rabbits, rats, and human subjects.

5. Discussion

The hardware described in this paper provides a useful tool for the simultaneous control of stimulus presentation and acquisition of classical conditioning data in both human and animal subjects. Using readily available components, the system is reliable, accurate, and convenient. There are a variety of ways to implement classical conditioning experiments and to instrument them for replicable use. The hardware system detailed here and the software for stimulus presentation and data acquisition and reduction described in the following paper (Akase et al., 1994) represent comprehensive and user-friendly solutions to the problems of running sizeable numbers of subjects in associative learning studies in a manner that provides consistent, comparable data within subjects from day-to-day and across both subjects and groups from session-to-session and cohort-to-cohort.

The complete system incorporates refinements and techniques we have developed over the years in the course of completing a large number of associative learning experiments. We have been using this system as described for the past 3 years to eyeblink condition rabbits, and with slight modifications to condition rats and humans in the same task. We have also assisted collaborators at different institutions in setting up the complete system as described in these papers, and their experience has been similar to ours with regard to the system's use. It has been relatively easy to obtain reliable eyeblink conditioning data, with consistent and replicable unconditioned response amplitudes and learning rates, from application to application. It has also been a much simpler process to train inexperienced students and technicians to successfully run the system on a daily basis than had been the norm with earlier systems. We describe the system for those who might be considering setting up associative learning

experiments as a new procedure in their laboratories, for those who may wish to use eyeblink conditioning as a powerful and reliable component in neuroscience laboratory courses, and for those who might wish to upgrade their current eyeblink conditioning apparatus to take advantage of the relatively stable, non-invasive methods described here.

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