

## Applications of multimedia computers and video mixing to neuroethology

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Received 14 October 1996; received in revised form 4 January 1997; accepted 25 January 1997

### Abstract

Inexpensive multimedia computers offer new possibilities for mixing video and computer images, videotaping these mixed images, and extracting quantitative data from videotape. In this paper we describe methods for mixing images from a video camera and a Macintosh computer display using chroma keying, and we describe a simple circuit for analog video mixing and frame-counting. We present three applications of these video mixing methods to our neurophysiological and behavioral research with awake, behaving animals. These technologies enhance accuracy, speed, and flexibility during experiments, by allowing us to record an information-rich videotape of the subject and state of the experimental apparatus. After the experiment, these technologies facilitate selecting and extracting quantitative data from the videotape for further analysis. The videotape is especially useful in resolving minor inconsistencies or incomplete information in the notes and data files that often arise during analysis of complex experiments. © 1997 Elsevier Science B.V.

*Keywords:* Animal tracking; Data synchronization; Screen-splitter; Behavioral monitoring; Mapping; Electric fish

### 1. Introduction

Video cameras and videotape recorders (VCRs) are powerful and readily available tools that in principle can capture and store information at extremely high data rates for long periods of times. Their use in experimental research has been limited, however, because extracting quantitative information from videotape and synchronizing the tape with off-camera events is generally difficult and expensive. Recent developments in multimedia personal computers and analog video multiplexers can ameliorate these problems, and thus expand the utility of video to the experimental neuroscientist.

Neuroethological experiments frequently require several different types of data to be acquired, synchronized with each other, and stored. For example, an experiment may generate physiological data stored on analog

tape and/or digitized to computer files, protocols stored in notebooks, tape recordings of experimenter's comments, photographs, etc. In addition, there are many instances when it is desirable to display on a single monitor, or record on a single videotape, the synchronized outputs of two video cameras. For example, in animal tracking experiments, the simultaneous views of two camera angles are necessary to determine positions in three dimensions.

During data analysis, synchronizing data stored in different media or on separate videotapes is often problematic, especially if the required temporal precision is substantially less than 1 s, or if the periods of experimental interest are sparse in long video recording sessions. For such analysis, it would be convenient to record on a single videotape information about both the subject and the state of the experimental apparatus, and images from multiple cameras. In this paper, we describe methods for mixing computer and video images, and for mixing images from two video cameras

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into a single video signal. We present three applications of this technology to our neurophysiological and behavioral research (Rasnow et al., 1993; Hartmann and Bower, 1996).

We first describe how the Apple Macintosh 660AV and 6100AV families of multimedia computers support live video, and in particular, how video can be viewed, annotated, and analyzed within virtually any commercial software package. These methods work even with programs that are not explicitly designed to support video, such as MATLAB (The Mathworks, Natick, MA) and Illustrator (Adobe Systems, Mountain View, CA). We have focused on these particular computers because they support the methods described here with stock hardware and require no programming. It may be considerably more difficult to implement these methods on other computer models and platforms.

We next describe a novel method for mixing the outputs of two video cameras into a single video image, which can then be recorded on standard videotape for later review and analysis. This simple, inexpensive circuit works equally well with PAL and NTSC video, and it also provides an output for running a field counter useful for data synchronization and time coding of the videotape.

Finally, we describe our use of video in three specific experimental applications: (1) we used chroma keying to mix computer and video images to create high-resolution maps of the electric potentials generated by weakly electric fish; (2) we used the analog video mixer to synchronize neurophysiological recordings with ongoing behaviors of the freely-moving rat; and (3) we combined chroma keying and the analog video mixer to study the electric fish's behavioral strategies for electrolocation. Although we present these multimedia techniques in the context of our own research, they are likely to have many other applications in experimental neuroscience.

## 2. Multimedia computers

The term 'multimedia computer' as used in this paper refers to computers capable of displaying and processing video images. The video input is generally digitized and written to a screen buffer using high-speed dedicated hardware. The screen buffer is in turn displayed on the computer monitor, also using dedicated hardware. Both the video and display hardware operate in parallel with the other computer subsystems, and thus do not substantially degrade performance of other computer tasks. The Macintosh computers we used have video outputs in addition to their video inputs, so the computer screen (including the video input displayed on it) could be recorded with any standard VCR.

A common use of multimedia computers is digitizing video frames and storing them to disk, or 'frame grabbing'. However, frame grabbing places high demands on many of the computer's subsystems. Since each full-resolution video frame contains over 300 000 pixels, digitally storing uncompressed video requires on the order of 10 million bytes per second to be transferred to disk (1 s of uncompressed NTSC video contains  $\approx 640$  by 480 pixels per frame, each pixel requires one or more bytes, and there are 30 frames per second. Values for PAL video are similar). Personal multimedia computers are thus often seriously limited in their frame capture rate, resolution, and storage capacity, and usually record video at lower quality than inexpensive VCRs. In the applications described here, instead of frame grabbing, we viewed video on the computer screen, and recorded the combined computer screen and video input to analog videotape (e.g., Fig. 2B). The same computer simultaneously controlled the experimental apparatus, which included demanding tasks such as unbuffered data acquisition at 288 000 16-bit samples per second (Rasnow, 1994).

It is also possible to add video capabilities to non-multimedia computers by installing plug-in cards. For example, we first experimented with multimedia techniques using a TV Producer card (Computer Friends, Portland, OR) in an Apple Macintosh II computer. The requisite capabilities of a video card for our applications include video input and output, and chroma keying.

### 2.1. Chroma keying and transparent video

In order to synchronously record events on video and the computer, the multimedia computer must combine two source images—the computer's normal display and the live video input—into one output image. In this section we describe various ways this mixing can be done. Frequently video is displayed in a rectangular window, and the computer image is displayed unaltered everywhere else. Chroma keying and transparent video are two alternatives for mixing the source images together on a pixel-by-pixel basis. Imagine the video and computer images displayed in separate layers or panes, like pages of a manuscript, with the video image below the computer image. In one variation of chroma keying, all pixels of the computer image that are a specific 'key' color (or shade of grey) are replaced with the underlying video pixel, i.e. the key color becomes transparent to the underlying video. The resulting output image can contain multiple and arbitrarily shaped regions of video and computer images. Chroma keying is frequently used in TV broadcast to superimpose text on video, weather reporters over weather maps, etc.

Chroma keying is just one example of 'alpha mixing', where each output value is computed by a weighted average of the source values (Jack, 1993):

output pixel =  $\alpha$ \* computer pixel + (1 -  $\alpha$ )\* video pixel

For chroma keying, the weights ( $\alpha$ , 1 -  $\alpha$ ) are either zero or one, depending on the color of the computer's pixel. Another alpha mixing rule called 'video transparency' is achieved by setting the weights to constants between zero and one. This results in both computer and video source images being visible and semi-transparent, similar to 'cross fading' transitions in cinema. Since both computer and video source images are attenuated by their respective weights, the contrast and saturation of each are proportionately reduced in the output image. This inherent image degradation with video transparency has made chroma keying the more frequent choice in our research.

## 2.2. Viewing video within any computer program

Alpha mixing video with the graphical output of computer programs provides many opportunities for novel data display and analysis. For example, one can annotate or trace video images in any graphics or drawing program by simply displaying the video with alpha mixing in a window and then drawing on the video image. When this is done, pixels in non-key colors appear to float over the underlying video (Fig. 2B). The saved file contains just the drawings and annotations, and not the underlying video. This is quicker and more efficient than the more conventional method for tracing a video image: frame grabbing the video to a file; loading the image file into a layer of a graphics program that supports multilayered drawing; tracing the image; and finally, deleting the frame grabbed image and its disk file.

Alpha mixing works with most computer programs, including those not explicitly designed to support video, because the computer and video images are combined low in the computer's display hierarchy, at the level of the screen buffer. Most computer programs maintain their graphical and display environments at more abstract or higher levels. If the screen buffer is configured for transparency or chroma keying, then parts of a program's display area may contain live video, without the program being 'aware' of it. In other words, the program's function and operation are unaffected, regardless of whether video is visible within its windows.

## 2.3. Macintosh 660AV, 840AV, 6100AV, 7100AV, and 8100AV screen buffers

Multimedia computers differ widely in how their screen buffers can be manipulated, and programming at this level tends to be highly specialized and difficult. We present here some methods for implementing chroma keying without programming, on the above mentioned computers. Note that the details of these methods are

unlikely to work without modifications on other computer models.

Transparent video is available with a public domain System Extension from Apple Computer called 'AV Digitizer Options'. Installing this file adds an 'AV Options' pop-up menu in video monitoring software (such as Apple's 'Video Monitor'). Selecting this menu displays a window with controls to set the weights ( $\alpha$ , 1 -  $\alpha$ ) from eight values between zero and one. Every pixel of the display is alpha mixed with the video. Therefore, video can be visible within any application window located over the video monitor window.

Our method for chroma keying takes advantage of a peculiarity of these computers' screen buffer implementation: with the computer displaying 256 colors, one of the colors is always 'key' or 100% transparent to video. We find this color by displaying the palette of all 256 colors over the video window, and looking for a missing color filled with the background video and surrounded by the other colors. We then fill the desired parts of the application window with this key color to view the underlying video. For example, from NIH Image 1.29 software (<http://rsb.info.nih.gov/nih-image>), we select the key color using the Color Picker tool with the 'CLUT' (color lookup table) window positioned over the video, and fill the main window using Image's Paint Bucket tool. Chroma keying with MATLAB 4.2 is described in Appendix A.

## 3. A simple analog video mixer

In addition to mixing video with computer images, there are many instances in neuroscience research when it is desirable to mix the synchronized outputs of two video cameras. The circuit in Fig. 1 combines images from two video cameras (color and/or black and white) into a single video output that can be viewed on any television and recorded on any VCR. This mixer can be built from inexpensive and readily-available electronic components, and works equally well with PAL or NTSC video. A printed circuit board layout for the circuit can be found in Appendix B. The two video cameras must be synchronized or 'genlocked' with each other for their images to be mixed, and therefore at least one of the cameras must have an external sync input. Professional video cameras generally have this input, as do inexpensive cameras used for surveillance or security systems. These latter cameras are also available with extended infrared and low light sensitivity, making them ideal for low- to no-light recording. Note that the mixer does not conform to the standard 'picture-in-picture' convention. Instead of a full image appearing in a window at reduced resolution, the user selects which portions of each camera's field of view appear in the mixed image at full resolution.

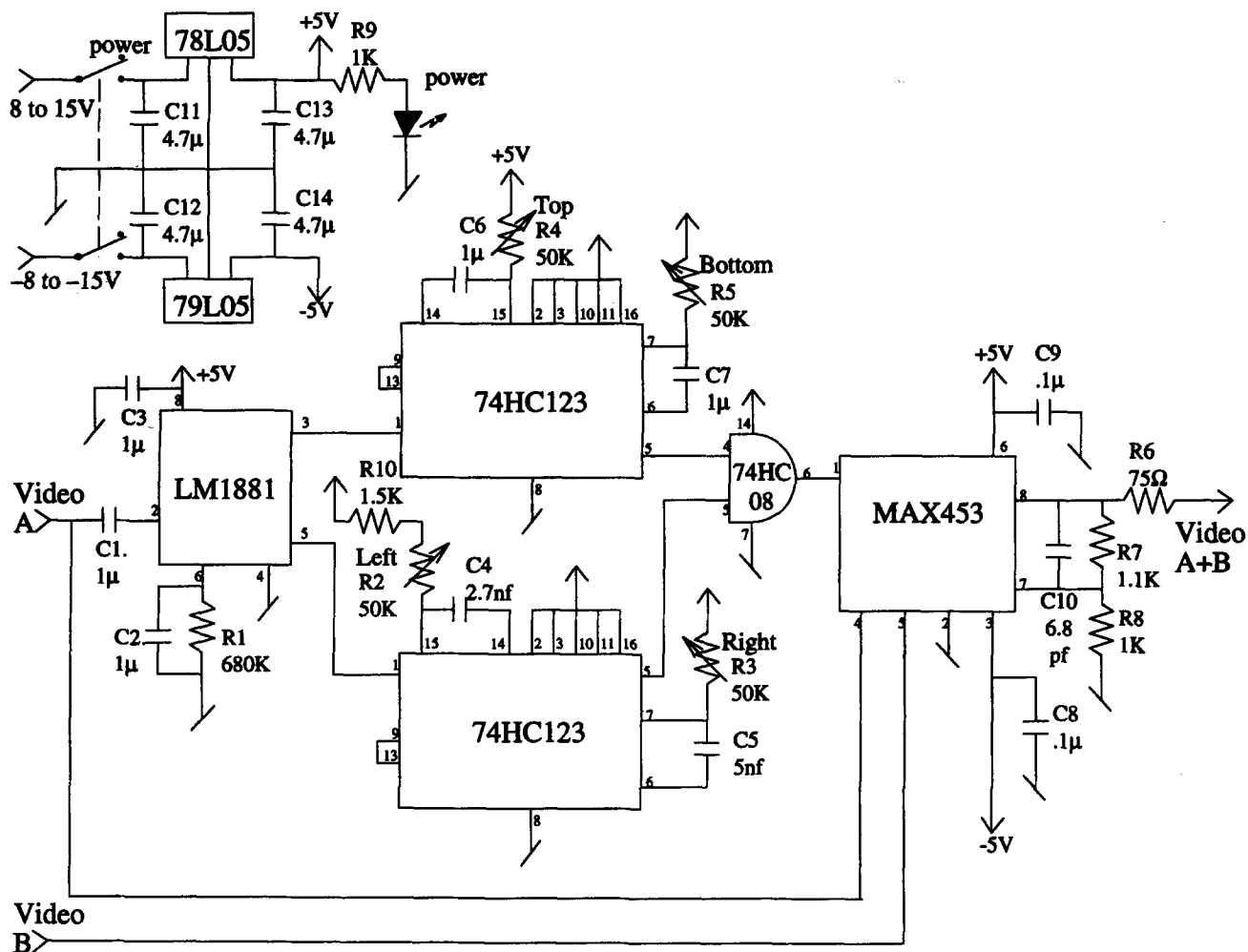


Fig. 1. Schematic diagram of the video multiplexer. The circuit in the top left is a voltage regulator providing  $\pm 5$  V for the mixer circuit below.

At the heart of the design is a MAX453 video amplifier and two channel multiplexer. This chip, by itself, is sufficient to mix two genlocked video signals; the remainder of the circuit is devoted to telling the MAX453 precisely when to switch between the two camera inputs. The composite video output of camera A feeds the external sync input of camera B and also the LM1881 video sync separator, which extracts vertical and horizontal sync pulses. The monostable multivibrators (74HC123) are triggered by these pulses and select the portions of each horizontal line from camera A that will be replaced with Camera B in the mixed image. The pulse time of the multivibrators is set by adjusting the potentiometers, which determine the size and position of the Camera B window in the mixed image. Each potentiometer adjusts one side of the window (left, right, top, bottom). The timing information from the horizontal and vertical multivibrators controls when the MAX453 multiplexer's output will switch between camera A and camera B. The MAX453 output can drive a 75Ω load directly, and thus can be sent directly to a standard VCR and/or TV monitor.

Vertical sync pulses from the LM1881 (pin 5) can be used to trigger a field counter LED display.

#### 4. Applications

In the following sections we briefly describe applications of chroma keying and video mixing to our ongoing studies with rats and weakly electric fish. First, we used chroma keying to accurately locate recording electrodes positioned on the body of a weakly electric fish. The goal of this experiment was to visualize and map the electric organ discharge potential as it varied in time and space across the fish's body (Rasnow et al., 1993; Rasnow, 1996). Second, we used the analog video mixing circuit to synchronize neurophysiological recordings with ongoing behaviors of freely-moving rats. We were specifically interested in examining the relationship between neural activity in the cerebellar granule cell layer and behaviors involving the whiskers and lip regions of the rat's face (Bower and Kassel, 1990; Hartmann and Bower, 1996). In the third applica-

tion we combined both technologies, digital chroma keying and analog video mixing, to study the behavioral strategies electric fish use to explore objects (Assad, 1997). Two orthogonal views of the fish's behavior were combined with the video mixer, and the fish's orientation and trajectory in three dimensions were determined using chroma keying.

#### *4.1. Mapping the electric potential on a weakly electric fish*

It is frequently necessary in experimental neuroscience to accurately locate stimulation or recording devices. For example, to map the electric potential on the skin of an immobilized weakly electric fish, it was necessary to record waveforms from electrodes sequentially positioned at hundreds of locations, accurately record the coordinates of each location, and infallibly associate each coordinate with its corresponding waveform (Rasnow et al., 1993). While mapping, it was also necessary to monitor the position of the fish, and if it moved at all, to return it precisely to its former position.

We achieved these goals by imaging the immobilized fish and electrode array with a stationary video camera, and displaying the live video on the computer monitor using chroma keying and NIH Image software (Fig. 2). We first traced the body profile with dots in a non-key color using the Measure Coordinates tool. If the fish moved during the experiment, these dots would become misaligned with the video image and be immediately apparent. We could then return the fish to its former position by realigning the video and body outline images. The body coordinates were also saved to a file and used to draw the body in subsequent figures and data analysis.

The fish's electric potential was measured with a flexible array of five electrodes pressed against the body. We measured each electrode position by clicking with Image's Measure Coordinates tool over the electrode tips, seen in the chroma keyed video. Note that the 500  $\mu\text{m}$  diameter electrode tips were generally too small to locate in single video frames. However, the lower noise and greater apparent resolution and contrast of live video allowed us to locate them. After measuring each tip position (which left an opaque dot floating over the video image), we switched applications from Image to MATLAB, and executed a script to digitize and record the amplified electrode signals with custom hardware and software (Rasnow, 1994). We then monitored on the computer screen the movement of the electrode array to its next location.

As the experiment progressed, the fish's body became covered with dots indicating where waveforms

had already been recorded, thereby permitting efficient and non-redundant sampling at additional points covering the fish's body (Fig. 2B). Upon completion of data acquisition, the waveforms recorded in MATLAB had to be combined with their respective locations, recorded in Image, to generate an electric field map. If there was doubt about the correspondence between these files (e.g. if there was an extra data point in the locations or waveforms files), it was a simple matter to review the experiment videotape, which contained a complete and synchronized record of all computer and video activity (along with two audio channels containing the experimenter's comments and the fish's electric organ discharge frequency). After additional digital signal processing in MATLAB, the waveforms were interpolated in space and displayed as pseudocolor movies of the fish's electric organ discharge. Interested readers can see the resulting Quicktime movies on our web pages (<http://www.bbb.caltech.edu/ElectricFish>).

#### *4.2. Synchronization of neural and behavioral data*

Correlating physiological data (e.g. electrocardiograms, electromyograms, or cellular activity) with an animal's behavior requires an accurate method for temporal synchronization. If in addition the animal behaviors of interest are initially not well classified, or ill-defined, the researcher needs to explore long periods of data correlating behavioral and neural events. However, it can be inconvenient and time-consuming to compare independent fiducial marks on physiological and video records, especially if periods of experimental interest are sparse in long video recording sessions. Finally, during careful frame-by-frame review of behavioral data it is convenient to have the corresponding physiological data visually superposed. We used the video mixer described in Section 3 to facilitate the classification of rat behaviors involving the lip and whisker regions (perioral structures), and to correlate these behaviors with cerebellar granule cell activity.

As shown in Fig. 3, we used the mixer to combine images from two video cameras: a consumer camcorder was used to monitor the rat's behavioral activity, and a black-and-white surveillance camera in another room was positioned in front of an oscilloscope displaying the neural data. Because the video mixer permits variable window sizes and positions, we placed the oscilloscope image in a region of the picture where the animal did not explore. Two LED counters were mounted above the oscilloscope display: one counted fields from the video mixer (thereby providing an accurate time-code identifying each video frame), and the second counted pulses

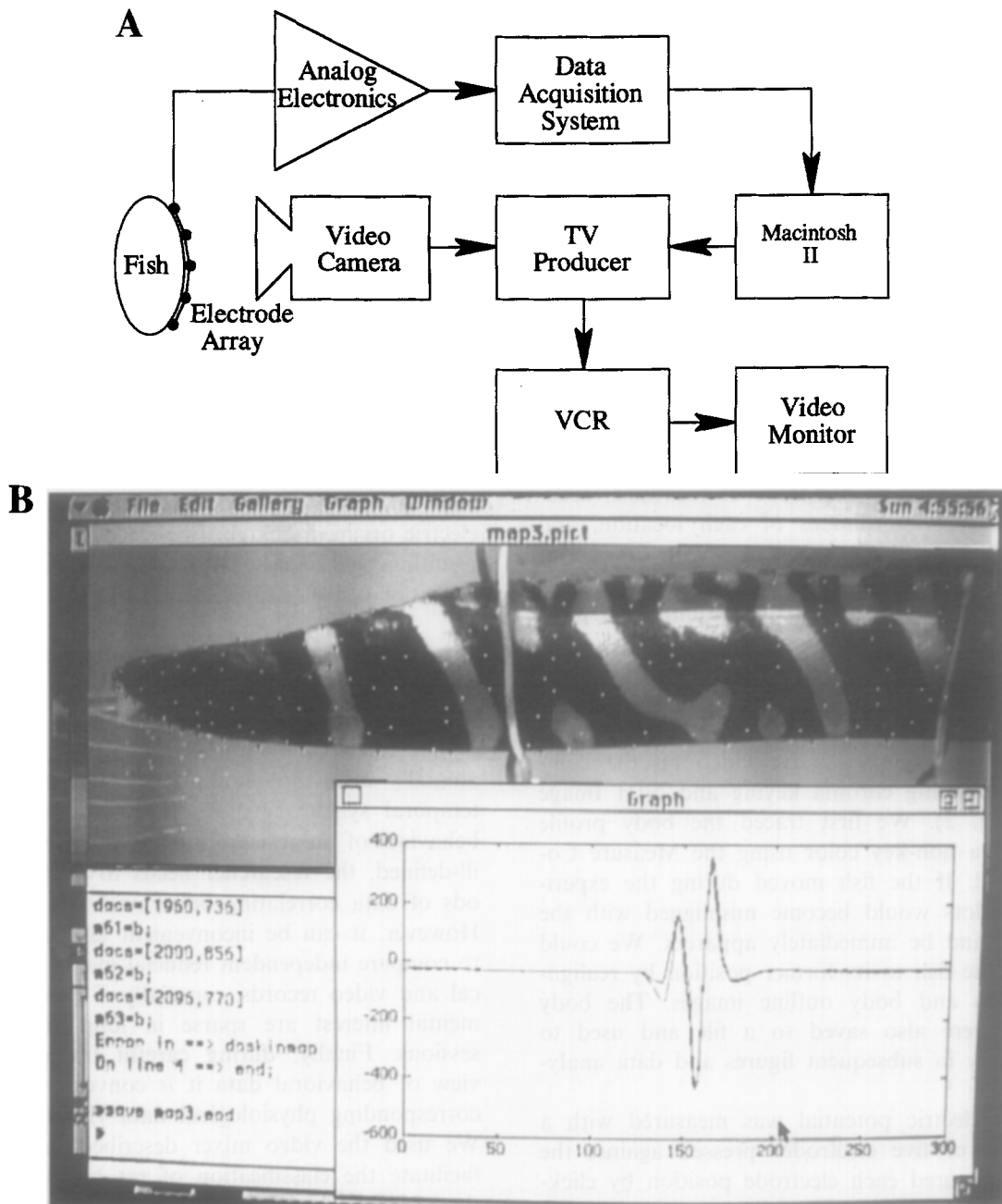


Fig. 2. A, Schematic of the apparatus for mapping the electric organ discharge of the weakly electric fish. The computer display is chroma keyed with video of the fish using a TV Producer card, and recorded on videotape. B, Photograph from videotape playback of the experiment. The NCSA Image window labeled 'map3.pict' was filled with the key color, revealing live video of the immobilized fish (*Gymnotus* sp.) and electrode array (far left). Prior electrode recording sites were drawn as dots in another color, which 'float' over the video. The fish's body was also outlined with dots in a different color. MATLAB 3.5, running in the foreground, displays waveforms sampled from the electrode array, and commands and diagnostics in its command window (lower left).

sent from the data acquisition system indicating the number of the open data file.

During the experiment, the video mixer allowed us to monitor the behaviors of the animal and the physiological data simultaneously, and in real time. After the experiment, we scanned through many hours of videotape examining correlated behavioral and neural activity. If a period of neural activity appeared particularly interesting, we could easily choose the correspond-

ing digitized physiological data file to examine the responses in greater detail. Because the behavioral and physiological data were visually superposed, we were able to correlate high activity levels with behaviors involving the rat's upper lip. By examining this relationship carefully, frame by frame, we discerned that the granule cell activity was not correlated directly with the movement of perioral structures, but rather with tactile input to these regions (Hartmann and Bower, 1996).

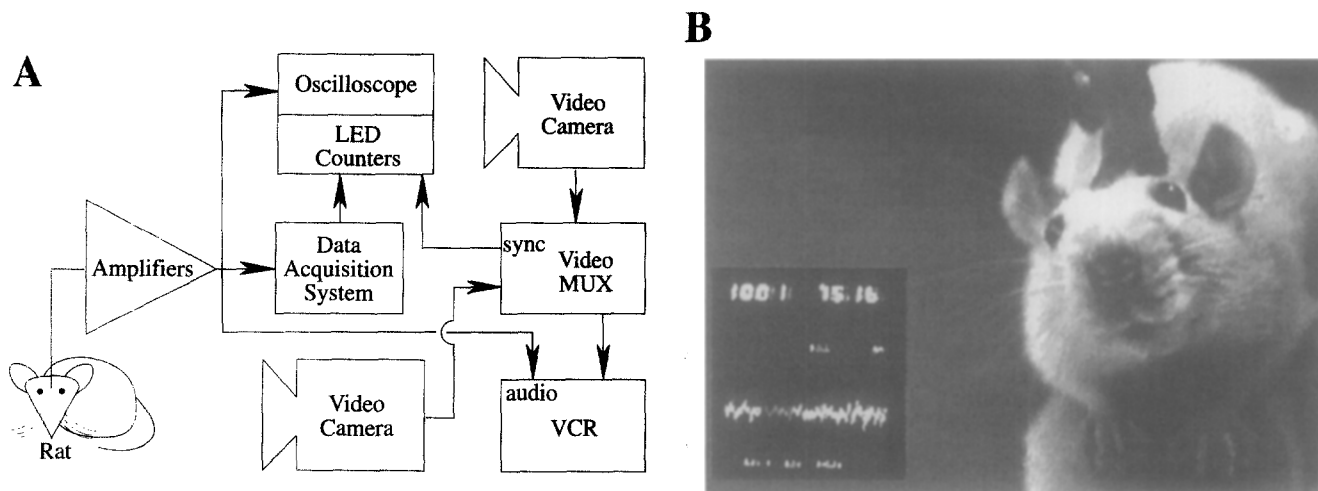


Fig. 3. A. Schematic of the apparatus for combining and synchronizing rat behavior and physiological data. Physiological data was also recorded on the audio channels (dashed line). B. Video frame showing the perioral structures of the rat with an oscilloscope trace of neural activity and video field counter.

#### 4.3. Reconstructing three-dimensional trajectories and body orientations during exploratory behavior

Quantitative measurements of animal behavior constitute another large class of neuroscience experiments. We studied exploratory behaviors by recording the movements of a weakly electric fish with two infrared-sensitive video cameras. One camera above the fish tank monitored movements in the  $x$ - $y$  plane, and the other, placed in front of the tank, monitored movements in the orthogonal  $x$ - $z$  plane. Video from both cameras was mixed, date and time were superimposed in a corner (Identi-Vision Systems V240T), and the combined image was recorded on a consumer VCR (Fig. 4).

We later analyzed the videotape on a Macintosh 6100AV multimedia computer, looking for interesting sequences of body positions and orientations to simulate the electric field (Assad et al., 1993; Assad, 1997). The video was visible in a MATLAB Figure window using chroma keying and Apple Video Monitor software (see Appendix A). We first overlaid coordinate system axes on the video using MATLAB's graphics functions. On the overhead view, we placed a three-dimensional coordinate axis using a flat (orthographic) projection along the  $z$ -axis. The side view suffered from perspective distortion (i.e. the fish's image became larger when it was nearer to the camera), which we compensated for with a perspective projection (MATLAB Reference Guide; Foley et al., 1990). The viewpoint and additional parameters defining this coordinate system were initially estimated from the camera location and lens focal length, and adjusted empirically by overlaying the coordinate axes with the tank edges seen in the video.

We rendered a three-dimensional 256 node surface model of the fish with non-key colors in these two coordinate systems overlaying the video. With graphical controls, we adjusted parameters to translate, rotate, and bend the model until it was in register with the video images of the fish (Fig. 4B). Aligning the video image and model directly proved more accurate and quicker than trying to orient the model from fiducial points on the fish's body. In fact, we could locate very few points consistently on the body, because of the low video image quality and the lack of visually distinct features on the dark fish.

#### 5. Conclusion

With its inherent high bandwidth and low cost, video is an underexploited tool for experimental neuroscience. Videotaping multiple video images, and mixing computer and video images have greatly enhanced our experimental capabilities. First, both chroma keying and video mixing can be used to synchronize images with high temporal resolution, necessary for animal tracking and for correlating different types of data. Second, because detailed experimental records are automatically captured on videotape, researchers can work faster and more flexibly during experiments, resulting in more data and less stress to the animal. After the experiment, chroma keying can be used to easily and accurately extract quantitative data from videotape directly into a variety of unmodified commercial programs. Finally, during data analysis, the mixed videotape images are invaluable in resolving and verifying any data inconsistencies or omissions in the written notes. Although we have described the use of multime-

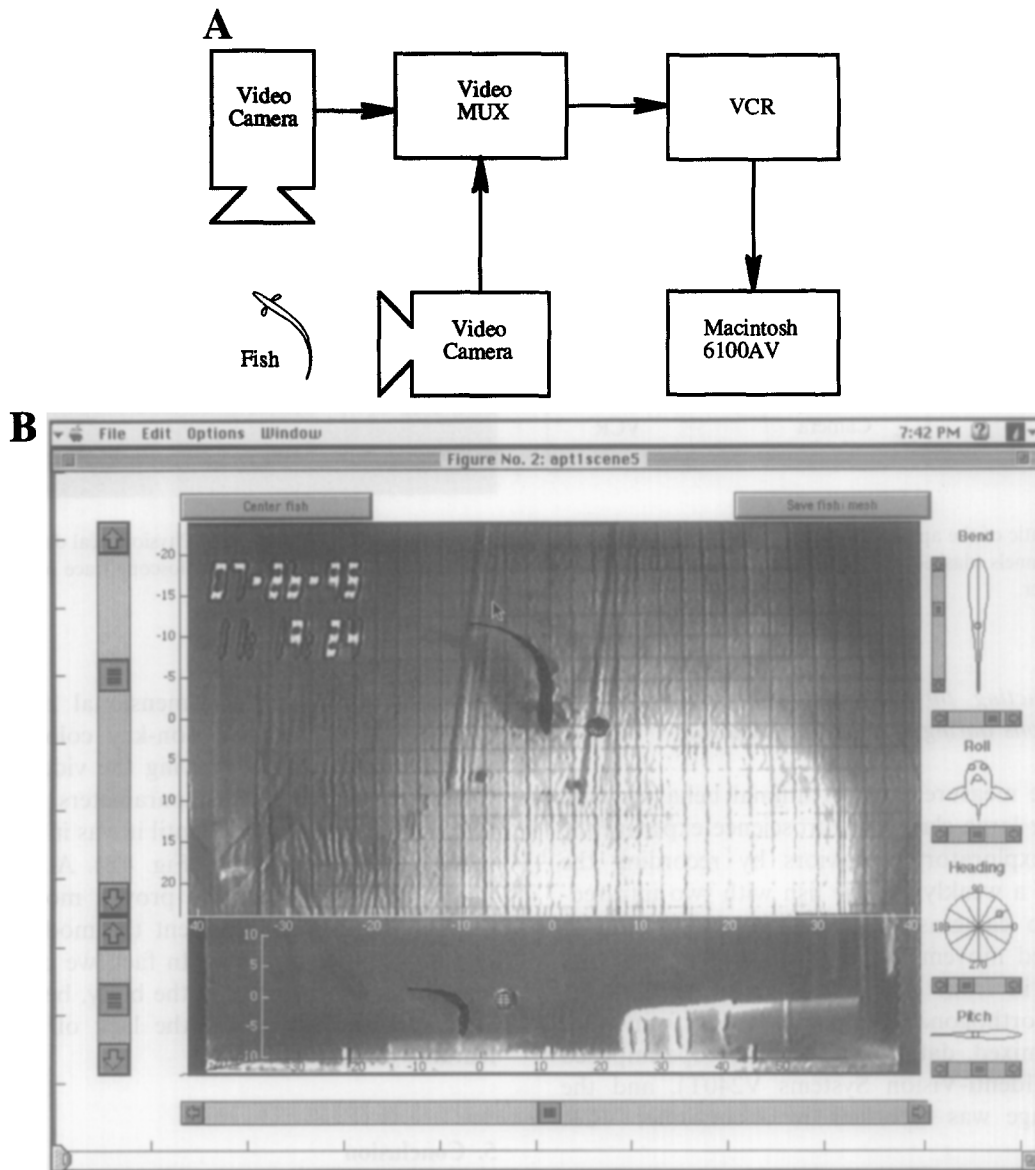


Fig. 4. A, Schematic of the apparatus for reconstructing trajectories and body orientations. Infrared-sensitive video cameras imaged the fish from two orthogonal angles. Their analog signals were mixed and recorded on a consumer VCR. B, Later, the videotape was viewed in a MATLAB figure window using chroma keying. A three-dimensional wireframe model of the fish's body and the object (black) was superimposed on the video and translated, rotated, and bent (by the controls around the window sides) to coincide with the fish's body in the two video views. Note that the video contrast and signal to noise ratio were inherently poor because the water absorbs infrared wavelengths, which were used for illumination without introducing visual cues.

dia and video mixer technologies in three specific experimental contexts (mapping, data synchronization, and tracking), there are many other experiments that could benefit from these methods.

#### Acknowledgements

This work was funded by NSF IBN-9319968. David Palmer provided valuable information about the Macintosh video system. Mike Walsh provided encouragement and valuable advice on building the video multiplexer.

#### Appendix A. Chroma Keying from MATLAB 4.2

The following code implements chroma keying on Macintosh 660AV and 6100AV families of computers running MATLAB 4.2 software. The computer must be configured to display 256 colors. Video monitor software (such as Apple Video Monitor) must be launched, and its window placed beneath the MATLAB Figure window prior to executing this script:

```
% make a palette of all 256 colors
c=hsv(256);
colormap(c);
```



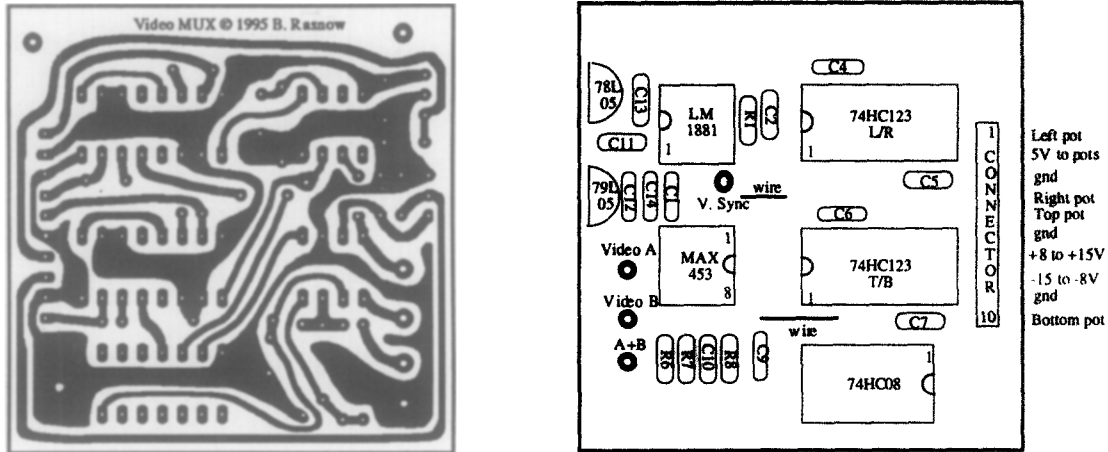


Fig. 5. Printed circuit board layout for the video mixer. The outer square is 5.6 cm.

```
% draw all colors in a large grid
set(gca,'units','normalized', ...
'position',[0 0 1 1]);
image(reshape(1:256, 16, 16));
% identify the key color.
disp('Place the Figure window and ...
the Video Monitor window and ...
click on the transparent color');
click=round(ginput(1));
colorIndex=(click(1)-1)*16+click(2);
% set the figure background to the key color-
clf;
set(gca,'Color',c(colorIndex,:));
```

#### Appendix B. Printed circuit-board layout for the video mixer

Fig. 5 shows the printed circuit board layout for the video mixer. The left image is the conductor layout pattern. This image can be photocopied onto transparency film, which can serve as a mask to expose positive photoresist coated single-sided circuit boards. The right image shows component locations and wiring connections.

#### References

- Assad C, Rasnow B, Bower JM. Numerical simulations of the electric organ discharge of weakly electric fish. In: Eckman FH, Bower JM, editors, *Computation and Neural Systems*. Boston: Kluwer Academic Publishers, 1992.
- Assad C. Electric field maps and boundary element simulations of electrolocation in weakly electric fish. PhD thesis, California Institute of Technology, 1997.
- Bower JM, Kassel J. Variability in tactile projection patterns to cerebellar folia crus IIa of the Norway rat. *J Comp Neurol* 1990;302:768–78.
- Foley JD, van Dam A, Feiner SK, Hughes SF. *Computer graphics principles and practice*. 2nd ed. Readings, MA: Addison-Wesley, 1990.
- Jack K. *Video Demystified. A handbook for the digital engineer*. Solana Beach, CA: HighText Publications, 1993.
- Hartmann MJ, Bower JM. Cerebellar granule cell layer activity correlates with the activity in primary somatosensory cortex in the awake, freely-moving rat. *Soc Neurosci Abstr* 1996;22:1200.
- Rasnow B, Assad C, Bower JM. Phase and amplitude maps of the electric organ discharge of the weakly electric fish *Apteronotus leptorhynchus*. *J Comp Physiol A* 1993;172:481–91.
- Rasnow, B. The electric field of a weakly electric fish. Ph.D. Thesis. University Microfilms International, MI: Ann Arbor, 1994
- Rasnow B. The effects of simple objects on the electric field of *Apteronotus*. *J Comp Physiol A* 1996;178:397–411.