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A cost-effective high-throughput digital system for observation and acquisition of animal behavioral data

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Running Head: A new rodent behavior observation system

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Abstract

We have designed and implemented a low-cost digital system for the recording of *in vivo* behavioral data in rodents using closed-circuit television (CCTV) cameras coupled to a digital acquisition system, allowing observation and recording of more than 10 animals simultaneously at a reduced cost compared to commercially available solutions. This new system has been validated using two experimental rodent models: one of chemically-induced seizures and one assessing appetite and feeding. Observational results showing comparable or improved levels of accuracy and observer consistency between this new system and traditional methods in these experimental models are presented. Advantages of the presented system over conventional analogue systems and commercially available digital system are discussed. Possible extensions to the presented system and applications to non-rodent studies are also proposed.

Introduction

The objective monitoring of animal behavior is necessary in diverse investigations within psychology and many other disciplines. However, the analysis of data acquired from such monitoring can be labor-intensive and time-consuming. Such observational analysis typically utilizes continuous video analysis to determine the frequency, duration and temporal distribution of various mutually-exclusive behavioral categories (e.g. locomotion, eating, drinking, grooming, resting), often via a computerized coding system (e.g. The Observer Video-Pro, Noldus, Netherlands). Furthermore, subsequent offline analysis of recorded behavior is often desirable to allow accurate analysis of complex, brief and/or high frequency behaviors. Offline methods also allows for blinding and analysis of data by observers. Such an approach can increase accuracy and/or be used to assess inter-observer consistency. Several options for video capture and storage systems are available, although these are frequently inefficient either in terms of time and effort or are prohibitively expensive. In the first instance, the use of consumer video cameras often requires laborious manual playback or separate offline encoding of recorded data; both of which are time-consuming. In the second instance, commercially available complete observation and recording systems can be expensive to purchase (~$10000). In either case, high-throughput recording of several animals simultaneously is either inconvenient or expensive.

Our laboratory investigates drug effects using a variety of voluntary and involuntary behaviors in rodents that include feeding (Williams, C. M., Rogers, P. J., & Kirkham, T. C. (1998); Williams, C. M., & Kirkham, T. C. (2002)) and, more recently, chemically-induced seizure studies. In order to remove the need for analogue video recording and to avoid incurring the cost of commercial, off-the-shelf observation systems, we have developed a cost-effective system for the monitoring, recording and subsequent storage of behavioral video data. This system employs closed-circuit television (CCTV) cameras attached via video capture cards to a personal computer (PC). Public domain camera and video management software is then used to control recording sessions. This system has been validated in two separate experimental models, can record data from up to 16 cameras even in low light (0.01 Lux (lx)), and yields digital video files directly to the PC hard disk for subsequent offline analysis. Additionally, it is versatile and easy to combine this system with other equipment to record additional data (e.g. to assess rodent food and water intake during video recording; The Drinking and Feeding Monitor; TSE, GmbH, Germany). Thus, the present system provides a cost-effective, efficient and reliable tool for the acquisition and storage of animal behavioral video data that is simple to assemble and use. Moreover, the flexibility of output formats provided by this system enables compatibility with a diverse range of offline analysis systems.

Methods

Experimental setups

All procedures complied with the UK Animals (Scientific Procedures) Act, 1986 and all data acquired were also used as part of ongoing research projects.

Chemically-induced seizures

Adult male (P>21; 60-100g) Wistar rats were injected intra-peritoneally (i.p.) with 80mg/kg PTZ (Sigma, UK) to induce seizures and rats were placed in the observation tanks immediately after injection as part of an investigation studying potential anticonvulsants. Recording commenced at this point to observe convulsant effects.

Rodent feeding

Food intake levels in twelve male rats (P>40, 200–250g on day one) were examined simultaneously using a food intake measurement system. A bank of twelve experimental cages containing a barred floor, Perspex rest area, feeding hopper and water bottles were used. At 10:00h, animals were weighed and placed in their individual experimental cages which contained standard lab chow (~30g) in computer weighed feeding hoppers. Food consumption was then monitored and behavior observed for 30 minutes. All feeding and behavioral data was then analyzed offline using The Observer Video-Pro behavioral analysis software.

Analogue acquisition system

Our former method for acquiring observational data employed conventional domestic video recorders (NV-DX110B, Panasonic, Japan) and was used here to provide a standard against which the new digital system could be compared and validated. In seizure model investigations, a maximum of three rats were separately placed in clear tanks (Savic 0129 Fauna box 6L; Aquatics Online, UK) approximately 1m away from three domestic portable video cameras and behavior recorded to video cassette. For feeding studies, the same cameras were positioned to view the cage from an angle similar to that used by the digital system (see below) at a distance of approximately 1m. For both experimental conditions, the recorded analogue video tapes were real-time captured to a PC via a USB-205 Audio/Video Grabber (Cypress Technology Co. Ltd, UK) and encoded to Motion Picture Experts Group 2 (MPEG-2) video. The resulting digitally encoded video was then analyzed using The Observer Video-Pro behavioral analysis software.

New digital acquisition system

For observation of chemically-induced seizures in rats, 5 clear tanks (Figure 1A i) were placed between black wooden dividers with CCTV cameras (TP-101BK, Topica, Taiwan; Figure 1A ii) mounted upon them (distance from tank to camera approximately 10cm).

For observation of feeding and behavior, CCTV cameras were positioned above each cage to allow an unimpeded view of rat behavior (distance from cage to camera approximately 10 cm; Figure 2A (*****)). To allow direct comparison of the analogue and digital acquisition systems in feeding and behavioral studies, in one instance, events in a single cage were recorded simultaneously by both systems. Data were analyzed offline using The Observer Video-Pro behavioral analysis software.

Validation of new digital acquisition system

Following acquisition of behavioral data from the analogue system and the new digital system, data were uploaded and coded offline using The Observer Video-Pro. Coding was performed twice on each piece of footage to allow reliability to be assessed. Intra-observer reliability analysis compares the consistency of scoring between pairs of observers assessing for both coding and timing errors. The reliability analysis generated a statistic (Cohen's Kappa [κ]) which is an overall measurement of agreement that is corrected for agreements that may occur by chance (Cohen, J., (1960)), and where a lower value indicates higher consistency.

Results

Requirements for the system

We identified several requirements for a new observation system to replace the use of analogue portable video cameras. These requirements included the ability to record simultaneously from many cameras, to either allow experiments with ≤16 animals or permit multiple-angle recordings of a smaller number of animals. The acquired video needed to be of a suitable format and sufficient quality for accurate offline analysis using The Observer Video-Pro behavioral analysis software. The cameras also needed short working distances to minimize space usage (compared with approximately 1m for analogue system cameras), to be easily mounted in a number of different ways and function at low light levels for observations under low or red light conditions (~ 4lx). Digital acquisition of data directly to PC hard disk was also a requirement in order to obviate any need for time-consuming real-time conversion of analogue video. The system described below fulfilled all these requirements and, importantly, was cost-effective and simple to assemble and use. A schematic of the digital observation and acquisition system is shown in Figure 3.

Observation system design

PC and video equipment

The components for two full systems were obtained. PCs were constructed using 3.16GHz Intel Core 2 Duo processors (Intel, USA), 2GB RAM and possessed 1TB hard drives to provide adequate processing speed and disk space during data acquisition. Two monitors (20”, Acer, Taiwan) allowed simultaneous viewing of all cameras. To minimize noise disruption to animals, low-volume fans were fitted to the PCs and fabric gauze attached to the interior of all computer case vents to limit infiltration of particulate matter. Two 120 frame per second (FPS) 8-port video capture cards (Bt787, Rockwell semiconductors, USA) were used to connect CCTV cameras to the video acquisition PC. Between 5 and 12 1/3” Sony CCD Black and White cameras (420TVL (television lines) 0.01lx, 12V DC) were connected to the computers via BNC cables, and powered with mains adapters. Cameras were distributed equally across the two capture cards to maximize the FPS capture rate.

PC operating system and software installation

The Open Source Mandriva Linux distribution (http://www.mandriva.com; http://www.linux.org) was installed on each PC based upon current ease of compatibility with the chosen hardware and acquisition software. However, any Linux-based system could be utilized for this purpose. The Open Source Zoneminder software (http://www.zoneminder.com, Triornis Ltd, Bristol, UK; Figures 4 and 5) was used for video monitoring and acquisition. All software used is freely available under the GNU Public License (GPL; http://www.gnu.org/copyleft/gpl.html), allowing free use, distribution and modification by the user. Zoneminder provides a web-based interface to underlying video camera hardware control (Figure 4) running on the Open Source Apache web server (http://www.apache.org), although other web servers could easily be used. On entry, this interface provides hashed authentication for local and remote users thereby securing access to stored data and video observation system control. Such login security allows users to monitor ongoing experiments on a separate computer away from the laboratory via local network or the internet. Following successful login, the user is presented with the principal control screen (Figure 4A) that provides a general overview of connected video devices, a summary of recorded “events” (a term Zoneminder uses to describe sections of recorded video, see below) and access to additional settings and controls. Video device control (record or monitor) and review of existing video records are accessed via this interface by clicking on the relevant device or event (Figure 4A). Recording by groups of devices can be started simultaneously using a single command, or can be started on a device-by-device basis. Additional settings and controls available via this interface include access, capture, encoding, bandwidth, video montage and video device cycling, allowing the user to setup and maintain the system through this single web-based frontend. The principal interface also allows web-based access to all existing video records (Figure 4B) including thumbnailed previews. This interface allows events to be replayed (at varying speeds), paused, compressed, securely uploaded and archived to alternative servers, deleted, encoded and downloaded to a local computer; batch operations on multiple event records are also possible via this interface. Acquired video and associated metadata are organized and maintained via a MySQL-based (http://www.mysql.com) database which the end user requires little, if any, interaction with since the web-based interface is used to call a number of Perl (http://www.perl.org) and other (e.g. Bash; http://www.gnu.org/software/bash) scripts that form part of the Zoneminder package and perform the necessary administrative and video/image conversion functions. Additional features that are also accessible via the web-based interface include motion-activated capture, acquisition FPS, continuous recording and unrecorded monitoring. It should also be noted that the modular and scripted nature of the Zoneminder system also allows more experienced users to easily write shell scripts to automate customized, complex tasks from the command line, if desired.

Video processing

In order to maintain high capture quality and frame rates from attached cameras, Zoneminder stores each frame as incrementally numbered Joint Photographic Experts Group (JPEG) format image files. Due to file number limitations in a given directory, long recordings (>10,000 images) required the division of such recordings into “events”, with the images comprising each event stored in a separate directory (Figure 4B). After completing a series of experimental recordings, individual frames within a given event may be rapidly converted to MPEG-2 format video using FFMPEG (http://ffmpeg.mplayerhq.hu) via the Zoneminder web interface. Alternatively, all captured events in a specified range can be converted with a single custom shell script:

```
# Usage: vid_gen.sh <starting event number> <finishing event number>
for event in `seq $1 $2`
do
/usr/lib/zm/bin/zmvideo.pl -o -r 1 -s 1 -e $event
done
```

Throughout this process, whether by automated shell script or Zoneminder web interface, detailed settings associated with video format (`-f` and `-F`), frame rate (`-r`), size (`-S`) and scale (`-s`) may be specified by the user. Use of the shell script allows the user not to be present during video conversion, which may last several hours if converting lengthy recordings (>15 hours). Thereafter, MPEG-2 encoded event videos may be manually concatenated into a single MPEG per animal per experiment:

```
cat [filename1] [filename2] ... > concatenated_mpeg.mpg
```

Alternatively, this process may be automated for a series of experiments via a simple shell script and alternative video output used (e.g. Xvid (http://www.xvid.org/) by means of Open Source encoders such as Mencoder (http://www.mplayerhq.hu/). Converted and concatenated video files may be then directly imported into The Observer Video-Pro (or alternative coding software) for review and coding.

Use of observation system to record *in vivo* rodent seizure activity and feeding behavior

The new observation system was successfully used to record PTZ-induced seizures in up to five rats simultaneously (figure 5). As described in Methods, 5 black wooden dividers were used to separate animal tanks as well as serve as camera mountings. To validate the system in this experimental model, the reliability of seizure scoring using The Observer Video-Pro was assessed for both the old analogue and new digital systems. Consistency of scoring was comparable using the analogue acquired video (κ = 0.91) and digital acquired video (κ = 0.92). The described system was also successfully used to record a group of twelve rats in individual cages for periods of seven hours and assess their feeding behavior during this time. To validate the system in this experimental model, an identical 30 minute piece of footage from the analogue and the digital system was coded. Consistency of scoring was improved using the digital acquired video ($\kappa = 0.94$) compared to the analogue acquired video ($\kappa = 0.86$).

Discussion

This study describes the successful design, implementation and validation of a new system for observing and recording *in vivo* behaviors in rodents. Use of this system has allowed higher throughput recording of greater numbers of animals than was previously possible in our laboratory.

As previously stated, the analysis of data acquired from behavioral investigations is highly dependent upon the accuracy and reliability of the coding conducted by one or more experimenters. The presented system not only allows for offline analysis of acquired footage which facilitates blinding and analysis of data by experimenters not necessarily involved with acquisition, but has also here been shown, by reliability testing, to produce comparable or indeed increased consistency of scoring of studied behaviors. Factors that increase the consistency and independence of scoring are critical for the production of reliable, high quality quantitative results in addition to being used to assess inter-analyzer consistency.

Moreover, rising costs associated with facilities for conducting behavioral investigations with animals, coupled with increased pressure for available space to be efficiently used, are also addressed by the presented system. Due to the low noise and networked nature of the presented acquisition system, it can be sited in the same room as the animals are housed (Figure 1A iii), while the short working distances of the CCTV cameras (which can be modified) allows a compact experimental area in comparison with the area required to use an equivalent number of domestic portable cameras. Such a setup permits either direct control of the acquisition system in investigations where the experimenter must be present at all times (e.g. seizure models) or matching levels of control from a remote location when the presence of the experimenter would disrupt the subjects (e.g. feeding studies). Secure remote viewing features also permit intermittent observation of experiments by other researchers without the need for potentially disruptive staff movements.

One complete system, as previously described, costs ~$1700; excluding tax. This specification included 12 cameras, all necessary connectors and cables, capture cards and the data acquisition PC. In comparison, to achieve the same result using domestic portable analogue video cameras would cost ~$2360 - $15100 (excluding tax; dependent upon quality and functionality). Furthermore, the necessity of laborious encoding of analogue to digital video files in real time would remain. Alternatively, use of commercially available systems that provide similar, albeit more limited, functionality as the presented system, have significantly higher purchase costs (~$9450). The specified components of such commercial systems are comparable or of a lower quality than those used to construct the presented system. Thus, the raised cost of commercial systems likely rests with the costs associated with proprietary control software and product support. Our use of freely available, Open Source software has permitted no software costs to be incurred whilst still utilizing tools that are widely used and robustly tested. Moreover, support for the Open Source operating system distribution and software is widely available and is often considered one of the most notable strengths of Open Source software. This comparably lower cost of ownership also encourages the testing and implementation of extensions to the described system for custom applications using additional Open Source packages. Such extensions include batch automation of video conversion, automated data backups, webcasting of data, image recognition, motion sensitive, audio and area capture recording.

We believe that the presented system aims to offer an easy to use and cost effective digital video observation solution. However, it should be noted that, as with any in-house system, careful consideration should be given to hardware specification prior to ordering and assembly of components as the limitations of a system is largely determined by the specific use to which it may be put. Particular considerations should include required disk space and speed, maximum number of cameras per capture card, and overall processing power of the PC. Additionally, organization of overnight batch encoding of acquired video footage is required as the processing overheads associated with encoding prevent simultaneous capture and encoding.

Beyond the observation and recording of rodent behavior, we would expect that this system, with or without the extensions described above, would be well-suited to other behavioral investigations that require unobtrusive recording from several angles (e.g. preferential looking paradigms, focus group studies and parent-child interaction). In conclusion, we believe that the presented system provides a cost-effective and highly functional solution to many of the challenges presented by behavioral studies.

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Figure 1. Use of the presented digital observation system in monitoring chemically-induced seizures. **A** Experimental setup for monitoring and recording of 5 rats simultaneously, showing (i) animal tank; (ii) camera mounted on divider and (iii) PC. **B**. Image of rat within monitoring tank prior to seizure induction.

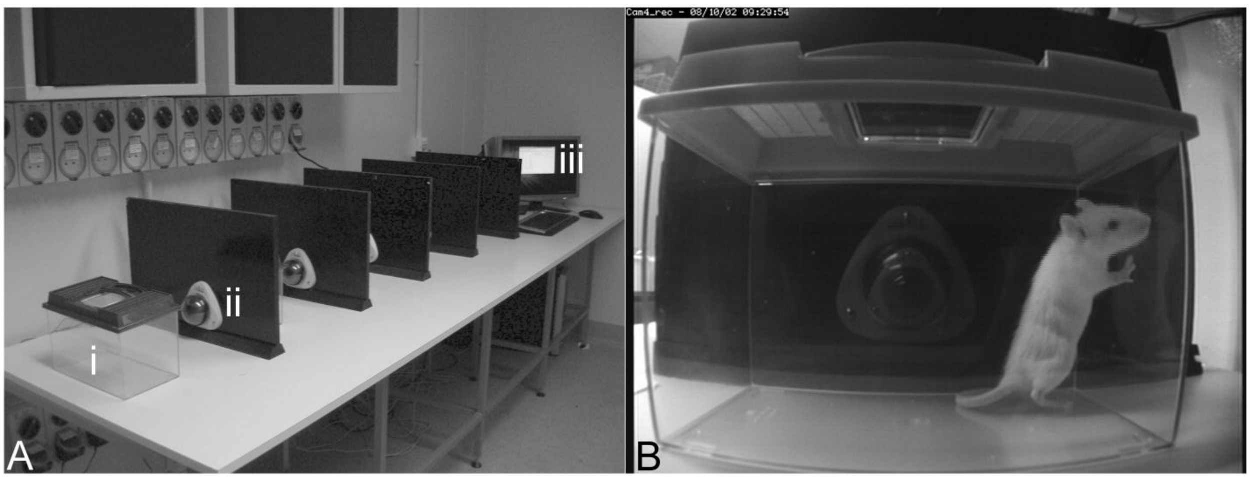

Figure 2. Use of the presented digital observation system for monitoring and recording rodent feeding behavior. **A**. Experimental setup and monitoring for 12 animals simultaneously, * denotes circled example camera. **B**. Still frame of rat acquired using the presented digital observation system in normal light. **C and D**. A comparison of still frames taken by the analogue acquisition system (**C**) and the presented observation system (**D**) under red light conditions (~ 4lx).

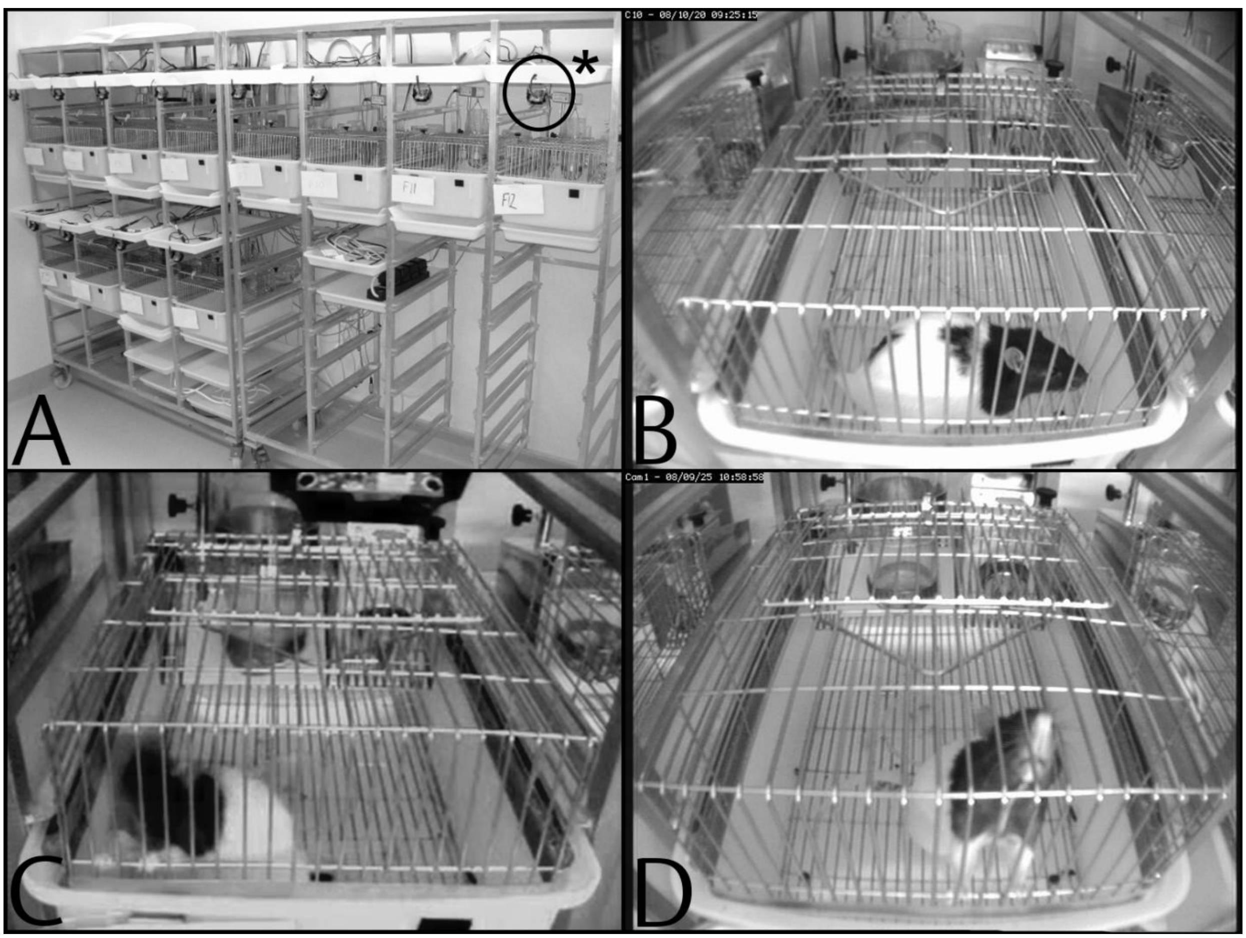

Figure 3. A schematic of the presented digital observation system. CCTV cameras (≤16) were connected to an acquisition and monitoring PC via video capture cards for display on dual monitors and simultaneous recording to hard disk. Zoneminder (ZM) software was used to control the cameras, monitoring and recording.

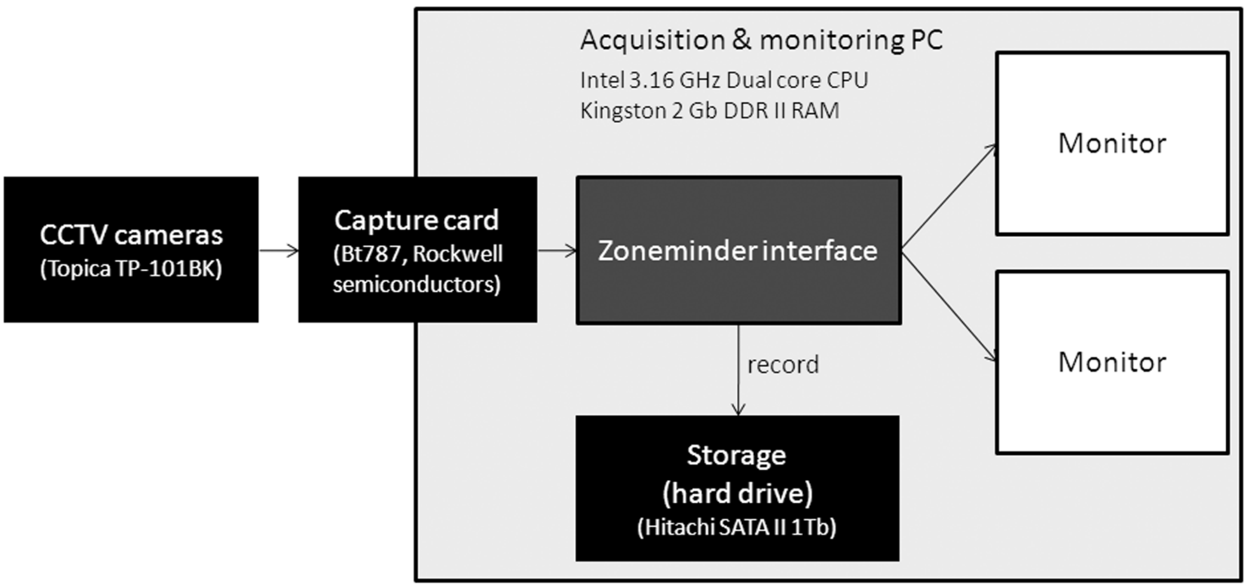

Figure 4. Zoneminder web interface. **A**. Control panel for 12 cameras. **B**. Zoneminder event log.

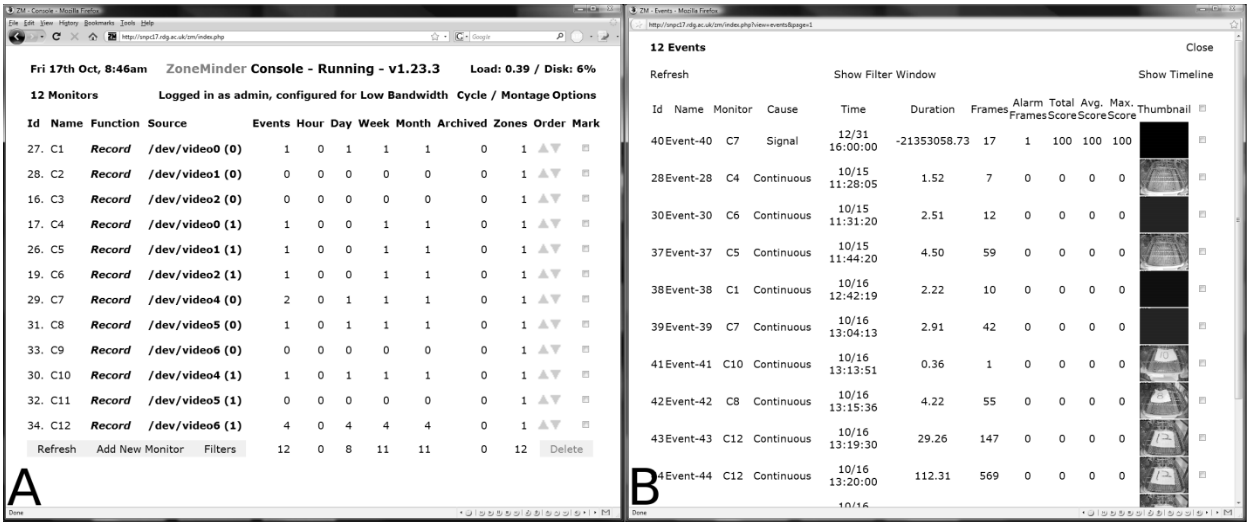

Figure 5. Simultaneously acquired monitor windows of rats prior to seizure induction using the presented digital observation system.