UNIVERSITY ANESTHESIA RESEARCH & DEVELOPMENT GROUP

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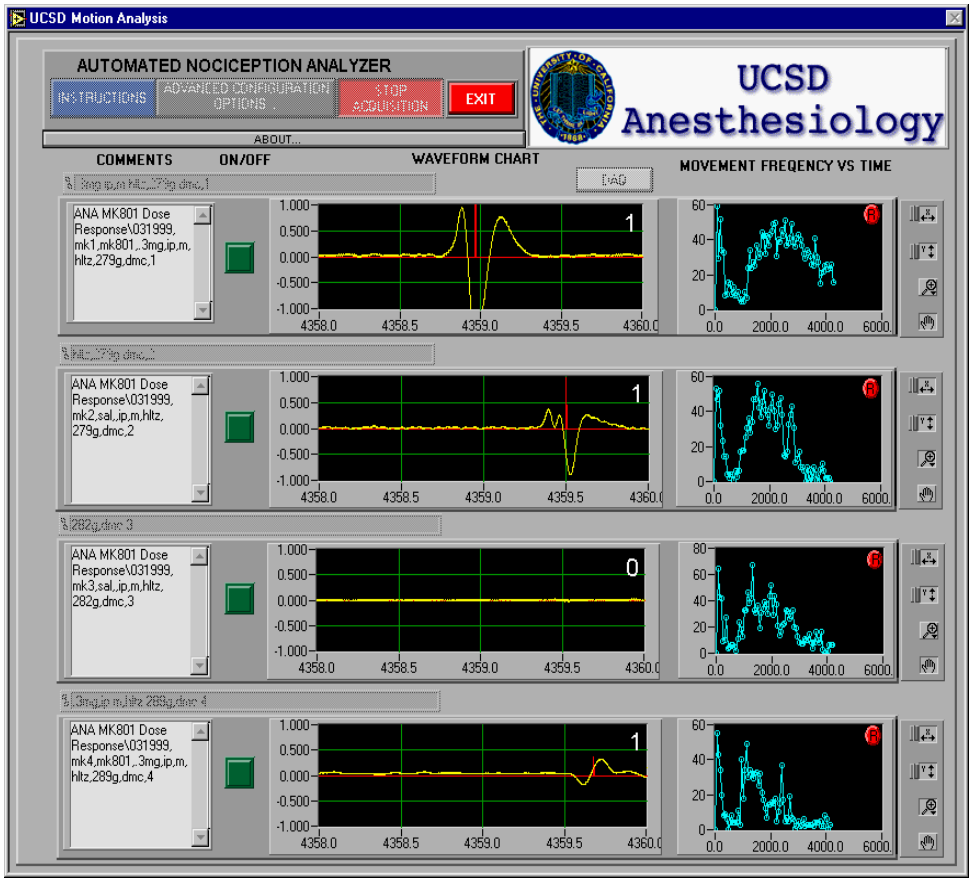
THE AUTOMATED NOCICEPTION ANALYZER

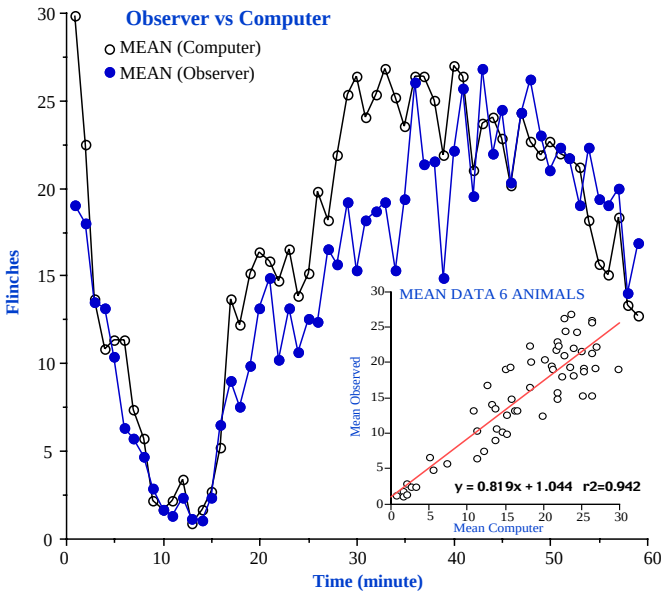
Injection of an irritant, such as formalin into the skin will result in the persistent activation of small sensory afferents and will evoke an organized constellation of responses that includes favoring, licking and flinching of the injected paw. The incidences of these nocisponsive behaviors occur in two phases: an initial first phase (phase 1, 0-10 min) and a delayed second phase (phase 2, 10-60 min). Current thinking emphasizes that the ongoing small afferent traffic generated by the irritant during phase 1 leads to a state of central sensitization during phase 2. The formalin test is thus a complex model that permits probing of the pharmacology and physiology of systems that are activated by tissue injury. In this regard, it is believed that the formalin test is a valid model for components of clinical pain associated with tissue injury. This places it in contrast to models such as the hot plate or tail flick where the response is evoked by an acute, transient stimulus that does not produce tissue injury. The formalin test is in wide use for mechanistic studies of nociception and for the evaluation of analgesic and anti-hyperalgesic agents. The magnitude of its impact can be appreciated by the fact that there were over 105 peer reviewed papers published between 1995 and 1999 using the formalin model in rats,.



From a practical standpoint, the test involves injecting one hind paw of the rodent with a small volume (10-50µL) of formalin (1-5%). The animal is observed to favor the paw and display periodic flinches in the injected limb. These flinches are counted at periodic intervals for typically up to an hour after injection. In normal practice, the observer is required to discriminate between a response or normal movement of the animal. From a negative stand point: the test requires considerable training to establish high "inter- observer" reliability; it is tedious, requiring uninterrupted attention on the part of the observer and finally it is labor intensive.

To address these issues, we have designed a device, which serves to detect the occurrence of paw flinches. This is accomplished by measuring the movement of a small metal band (0.5 grams) that is placed on the injected paw. Irritant is injected into the banded paw and the animal is placed without restraint inside the observation chamber over an electromagnetic detector system. The paw flinches are detected by the system

and counted automatically using a computer. At the end of the test, a file is written that contains the comment for each animal and the number of flinches per minute over time. The inset on the preceeding page, displays the screen showing the data for 4 rats that are just completing the 1 hour run. Each left panel is the animal file designator, the middle shows the real time analogue signal, while the right panel shows the minute flinch rate for the 60 minute run. This system has been extensively validated. As shown in the adjacent figure, plotting the flinch rate as measured concurrently by a trained human observer and the system revealed a correlation of r^2=0.942. The system thus permits the automated measurement of flinching behavior in 4 rats simultaneously. A single technician can monitor two systems concurrently. Depending upon study parameters this system can thus permit a single technician to undertake relatively large scale screening (6 runs/day x 8 rats/run). Moreover, the nature of the system means that the time required to train a technician and develop validating results is reduced to essentially a day. Finally, the period of counting flinches with the system is variable, but is nominally set at 1 minute. The human period of counting flinches is typically 5 minutes, leading to lost data.



The system as configured includes equipment and supplies to test 4 animals simultaneously and allow 4 more animals to acclimate over the period of testing. The equipment consists of, 4 detection devices, 8 clear plastic observation cylinders, signal acquisition module and BNC input interface with interconnecting cable, automated formalin testing, and data analysis software. (see picture below). Included with the analysis software, is a program for searching standardized file names, and moving data from these files into an Excel spreadsheet. The Excel spreadsheet calculates the count, mean, standard deviation, standard error and % maximum possible effect (MPE), on the control, drug, and dose data.

The computer is purchased by the customer as per our specifications and delivered to the Anesthesia Research Group. The hardware and software will be installed and the system tested. This process will not risk voiding the computer‘s warranty.

For more information and pricing concerning this instrument contact:
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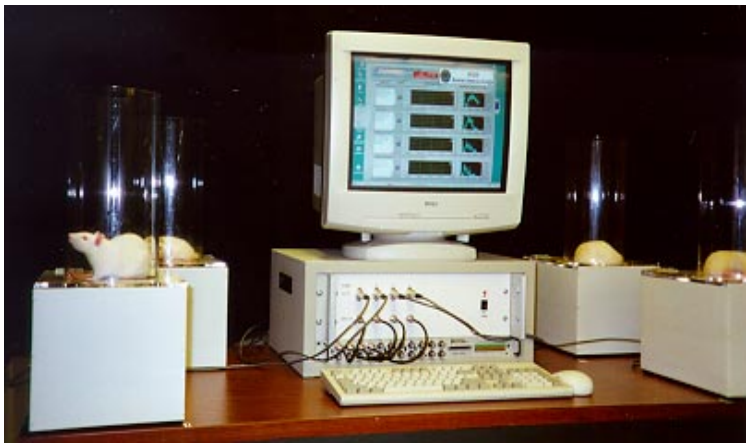

* Patent Pending