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Conditioned lick behavior and evoked responses using whisker twitches in head restrained rats

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Abstract

To examine whisker barrel evoked response potentials in chronically implanted rats during behavioral learning with very fast response times, rats must be calm while immobilized with their head restrained. We quantified their behaviors during training with an ethogram and measured each individual animals' progress over the training period. Once calm under restraint, rats were conditioned to differentiate between a reward and control whisker twitch, then provide a lick response when presented with the correct stimulus, rewarded by a drop of water. Rats produced the correct licking response (after reward whisker twitch), and learned not to lick after a control whisker was twitched. By implementing a high density 64 channel electrocorticogram (ECoG) electrode array, we mapped the barrel field of the somatosensory cortex with high spatial and temporal resolution during conditioned lick behaviors. In agreement with previous reports, we observe a larger evoked response after training, probably related to mechanisms of cortical plasticity.

Keywords

EEG; ECoG Electrode array; Restraint; Somatosensory cortex; Learning

1. Introduction

The size, shape and spatial distribution of cortical evoked responses can provide much information about the underlying mechanisms involved in sensory processing, especially during different behavioral and learning tasks. For example, the rat barrel cortex exhibits a variety of changes in evoked response potentials (ERP) during sensory processing, including large and small amplitude whisking and mechanical whisker stimulation [3,4,8,15,19,32,42,49,60]. The ERP also has a specific temporal pattern when the sensory stimulus contains meaning during conditioned learning which can change during training [1,26,35,38,42,50]. Additionally, the ERP amplitude is behaviorally state dependent [11,18,21,25,34], showing a

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higher amplitude during sleep and quiet resting and a lower amplitude during waking and REM sleep [25,36,39,40,45,53,58].

Many investigators have developed conditioned learning paradigms for freely moving animals [28,38], however the stimuli are coarse in nature and response times relatively slow. In order to test the behavioral consequences of detailed whisker learning with fast time resolution, animals must be restrained and be able to produce a fast response. For restrained rats, they can provide blinks or licks. Since blinking is traditionally a classical conditioned response, licking provides a better mechanism to examine an appropriate cognitive operant response. We developed a conditioned learning paradigm utilizing the lick response paired with whisker stimulation as the conditioned stimulus in order to simultaneously investigate the consequences of conditioned learning on spatial and temporal patterns of barrel cortex field potentials. To do this, rats were trained to be calm for long periods of time under head and body restraint, the electrical activation of the somatosensory barrel field was mapped with high spatial resolution to identify individual cortical columns, the whiskers were twitched in a well defined and consistent manner, and an appropriate learning paradigm was developed to test the animal's learning. Since some of our studies require the animal to sleep in addition to performance testing, the animal needed to be comfortable for long periods of time.

1.1 Behavioral training and preparation for restraint

For behavioral training involving both whisker stimulation and high channel count ERP recording, different methods of restraint may be used. Rats could be contained within a whole body wrap that completely encloses the animal's body within the restraint. Such devices include strait-jacket type wraps (PRO-RASNSM, ProMed-Tec, Bellingham, MA) and solid tubes [41]. Solid tube devices are often used to induce stressful conditions and can become hot and uncomfortable over long periods of time. Since the walls of the tube are not flexible, it does not allow the animal to make slight adjustments in body position if necessary for comfort.

The use of hanging wraps, or hammocks, has provided rats with more comfortable and relaxed conditions. They have been used in many studies involving awake, non-anesthetized, head-restrained rats to study sleep patterns, EEG activity, and pharmacological affects of drugs, and will typically allow the rat to remain calm in them for periods of time ranging from 3-7 hours after a one to two week adaptation period [2,9,23,24,46,52,55,56,57]. They have also been used for conditioned learning experiments where ERPs are recorded after a single whisker twitch paired with an aversive stimuli (electric current shock to the ear or tail) [42,60], or to study sensory-sensory conditioning to look at "learned" changes in neurons of barrel field of the rat by pairing two whiskers for stimulation [12,13]. One disadvantage of hammock techniques, however, is that the feet of the rat hang down through the hammock, possibly cutting off limb circulation, causing their feet to get cold and making them uncomfortable if they have to remain there for long periods. For our study, we tried both the hammock method and the straight-jacket wrap in order to make the animals comfortable. Since we required conditioned learning involving whisker twitching, performance testing, as well as sleep, we required a restraining mechanism that allowed the highest level of comfort possible.

Animal restraint is commonly used in studies involving electrophysiological analysis, with the goal of minimal stress when properly habituated [2,42,46,52,56]. However, attempts to behaviorally quantify animal stress has been limited, except in experiments where stressful conditions were the ultimate goal. For behavioral analyses, ethograms are widely accepted for quantifying behaviors portrayed in particular situations, such as open field, passive and active avoidance, consumatory behavior, or social behavior [14,16,17,20]. Depending on the study goals (e.g. stress and anxiety, drug abuse, influences of pharmacological manipulations, sleep/wake patterns, learning and memory) and the specific behavioral model used, different behavioral patterns and their categorization may be demonstrated by the animal, such as

sleeping, lying, rearing, walking, eating, drinking, and grooming [5,6,7,10,43]. For this study, the ethogram provided a method to quantify behavioral outcomes induced by immobilization and head-restraint, allowing us to calculate the level of tolerance the animal exhibited in these conditions. By tracking the behavioral responses, and gradually increasing the restraint time as the animal exhibited positive behaviors, long restraint times were achieved while the animal was calm.

1.2 Spatial mapping of the barrel field

High resolution maps of cortical activation are required to investigate the functional consequences of behavioral state and learning on individual cortical columns. Several studies have shown that surface field potentials using 100 μm or smaller electrodes from rodent brain can effectively map the representations of cortical activation, EEG and surface field potentials with high spatial and temporal resolution [27,32]. In order to achieve the best localization, electrodes need to be placed close to the tissue: either directly in contact with the tissue surface, or penetrating into the tissue. Our arrays were developed for chronic/awake preparations, and sit on the cortical surface to minimize tissue damage for long term recordings. Such multi-electrode arrays can record the spatial and temporal organization of both spontaneous and evoked electrical activity on the surface of the whisker barrel cortex, and have also been used to investigate high frequency oscillations [15,31,37]. Because the dura is relatively thin in rodents, high resolution is still feasible through surface recordings, enhanced by the use of a 64-electrode array which covers the barrel field and allow us the possibility to record from a single barrel.

1.3 Parameters for whisker stimulation

Vibrissae must be stimulated within specific parameters in order to achieve highest amplitude ERP possible. Many devices have been used in order to stimulate whiskers, including piezoelectric devices, solenoids, and speakers, all with some kind of armature placed approximately 5-10mm from the base of the whisker that is either touching or glued to the whisker(s), or involves a tube that the whisker can be inserted into, displacing the whisker in a ventrodorsal direction while avoiding touching other whiskers [8,30,32,33,42,60]. Stimulators can be attached to whisker groups to create a more widespread response, or to individual whiskers for a more localized response [8,33]. Piezoelectric devices deliver the fastest pulses with relatively low noise associated with each twitch, but are only able to exert short distance twitches (<200 μm). We developed a solenoid twitcher by wrapping magnet wire around a glass pipette tube in which we placed a permanent magnet coupled to a hooked wire. In this way, we deliver 1-2 mm twitches over 2 ms with quiet operation.

1.4 Conditioned learning model

Due to the fact that our procedure requires immobilization and head restraint, the most convenient model of conditioned learning is a licking response paradigm, where reinforcement with a drop of liquid is used as a reward, and the conditioned stimulus is the whisker twitch. The conditioned licking response to whisker stimulation could be used in the following situations: 1. single whisker stimulation in classical conditioned paradigm. 2. Two whisker discrimination in classical conditioned lick/no lick paradigm. 3. Two whisker discrimination in operant conditioned lick/no lick paradigm possibly with lever pressing for the water reinforcement [59].

The licking response has been used in previous studies of operant conditioning where free moving rats receive a water reward for performing progressive ratio licking (where increasing numbers of responses are required for successive reinforcements) or for distinguishing between amplitude modulated sounds where one was rewarded and one was not [44,48]. Studies in mice involve conditioned learning in response to vibrissal stimulation paired with either aversive

stimulation (mild electric tail shock) or a food/water reward in order to study the appetitive response [29,51]. However, we required fine control over individual whiskers, thus we could not use freely moving preparations or the brush technique of stimulating multiple whiskers at a time.

In the present study, we established a low stress method to train an awake, immobilized and head-restrained rat to lick in response to an individual whisker twitch and discriminate between a reward and a control whisker as we simultaneously mapped the cortical barrel field with high spatial resolution.

2. Methods

2.1 Training procedures

Experiments were initially carried out on 24 adult female Sprague-Dawley rats weighting 300-350 g, leading up to five rats that participated in all aspects of the experiment. All procedures were carried out in accordance with the “Guide for the Care and Use of Laboratory Animals” and were approved by the Washington State University Animal Care and Use Committee. Rats were habituated to immobilized conditions in both wraps and in hanging wraps (hammocks) 2-3 weeks before the surgery with incremental increases in time, starting at 5 min and continuing up to 45 min daily. They were rewarded with treats (sweetened multi-grain cereal) after each session. The aim of the pre-training was to achieve long term immobilization while reducing the stress of the animal.

We created an ethogram procedure to evaluate each animal’s ability to tolerate restraint. The chart we created allowed us to record behavioral manifestations relevant to our study for every 30 second period throughout training. Each behavior was categorized as “good” or “bad”, therefore providing a way to differentiate between quiet and active behaving animals (Figure 1). Rats were kept in the wrap for progressive time periods, unless they started to show obvious signs of extreme distress (restlessness, tearing etc.). The data collected by the ethogram allowed us to calculate the percentage of good behavior and plot the progress of training over time.

For restraint, we used both hammocks and straight-jacket type wraps with modifications to commercially available versions (PRO-RASNSM, ProMed-Tec, Bellingham, MA) to better fit our needs. The home-designed wraps (Figure 2A) were smaller to create a tighter fit around the rats, keeping them from wiggling extensively. They had altered Velcro (Velcro Industries B.V., Curacao, Netherlands Antilles) placement to make them more adjustable for each rat, as well as a modified Velcro “collar” that was internal instead of external, where the Velcro could wrap around the neck and be tucked inside of the wrap, providing a tighter fit around the neck with improved head motion restriction (Figure 2B). In addition to changing the jacket itself, we utilized a piece of felt to wrap the rat in prior to wrapping, which allowed for more comfortable conditions. The home-designed hammocks (Figure 2C) consisted of a continuous sheet bottom with leg holes and a laced top. A trapezoidal piece of material, with the smaller end by the head and the larger end at the rear of the rat, allowed for a tighter fit around the neck and improved the animal’s immobility (Figure 2D). Using the ethogram procedure, we assessed several different restraining mechanisms and chose the wraps as the best method of restraint because the animals were more comfortable and exhibited fewer bad behaviors.

2.2 Surgery procedures

To implant chronic electrodes, all animals were anesthetized with a short exposure to isoflurane and then brought to surgical levels with intramuscular injections of ketamine HCl (100 mg/kg) and xylazine (10 mg/kg), maintained by additional doses every hour throughout surgery such that the corneal reflex and eyeblink could barely be elicited. Body temperature was maintained

using a regulated heating pad and EKG rate was monitored. Lactated Ringer was injected subcutaneously and ointment was placed over eyes to prevent dehydration.

Two stainless steel ground screw electrodes were placed bilaterally 1 mm caudal and 1 mm lateral to the lambda-midline intersection (Figure 3). Screws electrodes were also placed over the left parietal and frontal cortices. Additional screws were set bilaterally in parietal and frontal areas for anchoring purposes in order to prevent the head-stage from moving or coming off during head restraint in later procedures. Two multi-stranded stainless steel wires were implanted subcutaneously near the low boundary of the rib cage symmetrically for EKG recording and two electrodes were implanted bilaterally into the neck muscles to record EMG activity. A unilateral craniotomy was performed to expose a 5×5 mm region of barrel cortex in the right hemisphere where the 64 (8×8) channel electrode array (with individual electrode diameters of 250 μ m, spaced 400 μ m apart) was placed. The leads from occipital ground screws, parietal and frontal screw electrodes, EKG and EMG wires were soldered to the sockets of the 64-electrode matrix. The construction was strengthened to the skull with acrylic dental cement, creating a strong single unit. A short piece of square aluminum was positioned posterior to the electrode construction to serve as dimples for painless stereotaxic restraint during training and was strengthened by dental acrylic. The scalp incision was closed and covered with a broad-spectrum antibiotic ointment. Analgesic Flunixin Meglumine 1.1 mg/Kg and antibiotic Gentomicin 5 mg/Kg were injected after the surgery and additionally for 2 days post-surgery. Rats were allowed to recover for 1 week after surgery before starting the conditioned training.

2.3 Conditioned learning

Rats were re-accustomed to restraint and experimental conditions after recovery from surgery until they were calm under these conditions. They were water deprived for 12-14 hours prior to any experimentation, and ½ hour after being returned to their cage to increase motivation for obtaining a water reward. Food was provided *ad libitum*. The rats were trained to lick from a lickometer, which had a spout driven by a solenoid valve that delivered approximately 0.02 ml of water for each correct response. Licking movements, detected by the rat's tongue crossing an infrared beam of light, were collected by the computer as they occurred (Figure 4).

ERP mapping of the recorded field was done to localize the barrel cortex under the array and determine the best whiskers to be twitched. Whisker mapping was performed 3 times, averaging ERPs for 50 stimuli for each whisker. Once the best pair of whiskers was determined, training for the conditioned response began. Conditioned training protocol involved stimulation of the whisker pair, one designated as the “reward” (conditioned stimulus), as it was rewarded with the delivery of water reinforcement (US), and the other as a “control”, as no reward followed stimulation. The stimuli (whisker twitches) were presented such that they were not too frequent, so that they remained separate events for the animal and not too slow in order to provide better consolidation of memory traces and to avoid shifts of the animal's state toward sleep. Whiskers were inserted into the solenoid hook and deflected at an amplitude of 1 mm over 2 ms at a position 1-2 mm from the whisker base with the whisker positioned upward. Whiskers were stimulated in random order with inter-stimulus intervals of 10-15 seconds. Conditioned learning usually lasted about 30 minutes per session and continued daily for 2-3 weeks. The conditioned response was considered stable after reaching 80% correct response to stimulus. We used peri-stimulus histograms to demonstrate the success of learning, plotted with 50 ms resolution. The first lick occurring within 2 seconds of stimulation was considered a valid response to stimulus, though once trained, animals typically responded within 200 ms.

2.4 EEG recording and data analysis

EEG from 64 channels, neck EMG, EKG, respiration were filtered between 0.1 Hz and 3.2kHz, amplified by a factor of 1000, and sampled at a rate of 20 kHz. Post-hoc analysis included triggered averaging with stimuli and licking events. For each channel of the electrode array, a topographical map was generated of electrical potential at the peak of the stimulus evoked response. Whisker barrels were localized by identifying the peak electrical response within the array. To assess the effect of conditioned learning on the cortical evoked responses, signals were compared between pre-training and post-training for lick responsiveness.

3. Results

3.1 Ethogram results

The use of ethograms allowed us to monitor and record various behavioral manifestations seen during restraint training. The chart also allowed us to differentiate between the calm versus active behaving rats under restraint conditions and allowed us to select for desirable behaviors, which helped predict success of the conditioned learning. By recording behavior every 30 seconds, we were able to quantify results for each session as well as chart progress over time for each individual. The data also enabled us to compare individuals quantitatively instead of relying on subjectivity.

At the beginning of training, the ratio of good behavior to bad was low and a lot of variance was seen among the individual rats (Figure 5A). As training progressed and the time in the wrap increased, the ratio of good behavior quickly increased and stabilized between 80-100% after about 30 days of training. This demonstrates that the rats were acclimating to restraint and improved over time. It is worth noting, however, that part of the decrease in variability over time is due to the fact that we selected the better behaved rats, and rats that had perpetually “bad” behavior under restraint conditions were excluded because we would not be able to use them for further experiments. From an initial population of 24 animals on day 1, only 5 remained in the study at day 30 (Figure 5B).

The training length, or time in wrap, produced a sigmoid shaped graph. Initially, the rats were all relatively active under restraint, sessions were short, and little variability was seen. This was followed by a rapid improvement as rats acclimated to restraint conditions or were excluded from the study. Rats showed a significant loss of weight of only one gram ($p < 0.01$) throughout the course of training from week to week, attesting that animals experienced minimal stress under restraint.

3.2 Behavioral indices of learning

Upon learning the conditioned task, rats were able to differentiate between the reward whisker stimulation and that of the control, and predict the presentation of the reward (water) as seen by a licking response. Figure 6 shows a single response immediately pre- and post- stimulation, and the subsequent events; the reward stimuli was followed by several licks, whereas the control stimuli produced no lick response. The evoked response can be seen in each case. Figure 7 shows the progress of learning throughout the course of the initial 4 days of conditioned training. On day 1, the animal showed licking response to both reward and control stimulation and there was a wide distribution of time in which the response occurred. By day 2, licks were still seen after both reward and control stimulation, but the distribution decreased as more licks were seen immediately following stimulation, particularly in the case of the reward stimulation. Fewer responses were seen after control stimulation by day 3, with a delayed response after the reward stimulation. By day 4, no licking occurred after control stimulation, and most of the licking occurred immediately following the reward stimulation.

3.3 Mapping

We identified cortical columns corresponding to two different whiskers using our 64 channel electrode array. The location of each cortical column was mapped by generating a 3D surface map of the electrical potentials (Figure 8A), where the peak amplitude in the map corresponds to the location of the whisker barrel associated with that whisker. This enabled us to plot the surface evoked response potential over time from the channel located directly over the corresponding whisker (Figure 8B).

3.4 Conditioning dependent ERP parameters

Evoked responses were recorded throughout lick training, showing larger amplitudes one week after the training commenced. Examples from two animals are shown in Figure 9. All components of the ERP increased, including the P1, N1, and P2, exhibiting a $25\pm 8\%$ increase in the evoked response amplitude (Mann Whitney U Test, $p < 0.05$).

4. Discussion

The present study provides several methods to overcome significant challenges induced by restraint and head immobilization, which are necessary for studies of cortical evoked responses during conditioned learning. To reduce the inevitable stress reaction caused by restraint, we used a pre-training procedure in order to adapt the rat gradually to immobilization. We started the rats out with 5 minutes in the wrap, and subsequently gave them a treat (sweetened multi-grain cereal) when returned to their home cage, so that they might build a positive association between the wrapping and reward. This was reinforced by consistently providing a treat upon returning to their home cage post-training. Increasing the restraint time by 5 minutes a day gave the animals sufficient time to acclimate to long restraint periods. As restraint time increased, the signs of stress caused by the procedure decreased, and the animal slowly became accustomed to being able to remain in the wrap for 1-2 hours. Rats were frequently able to remain still for long periods of time, and even in some cases, were able to fall asleep. In addition, none of the rats experienced significant amounts of weight loss over the course of the experiment, showing that they did not undergo high levels of stress due to the restraint.

Not all rats became accustomed to immobilization, however. Only 20-30% of the rats learned to withstand the long periods of restraint without showing significant signs of stress. Therefore, we used the ethogram protocol to select the most well-behaved rats and exclude the poorly behaved rats. Specific behavioral manifestations were recorded every 30 seconds and qualified as “good” or “bad”, which enabled us to look back at a rat’s training records to obtain a general impression of the rat’s performance for each session. Because our study required that we select for calm rats, our results may be skewed, as we are excluding a particular “personality” type, namely the active rats. Others have attempted restraint protocols such as this, however for more limited time periods and often not requiring sleep [2,13,42].

A high resolution electrode array was required for adequate whisker barrel mapping, allowing better spatial localization of evoked responses within the rat barrel cortex. While the present study did not obtain recordings from individual neurons [47] our 64-channel electrode array implanted on the surface of the cortex provided high resolution maps of the somatosensory field potentials with the possibility of recording population action potentials [32]. For the purpose of our study, the 64-channel electrode array allowed us to investigate the evoked response in individual barrels, specifically those corresponding to the reward and control whiskers.

In order to refine these procedures, consistency seems to be critical, including daily rat training, the time of day that training occurs, and ensuring that the same trainer works with a given rat.

To increase motivation of the rats to perform the licking task, we see improvement with 24 hour water restriction to a total of 100 ml/kg water each day [22,54]. When rats are water restricted for multiple days in a row, we obtain even faster learning involving licking for a water reward.

Surface mapping provides an advantage in that it enables a detailed analysis of how individual cortical columns respond to learned behaviors. For example, as reported earlier, evoked responses are larger after training [1,26,35,38,42,50]. In summary, we developed a protocol for animal restraint, recording, and conditioned learning that can be used for complex behavioral paradigms that require high resolution neural recordings along with fine grained resolution for stimulus parameters and behavioral responses. By using the chronically implanted ECoG electrode array, the consequences of learning can be studied within individual cortical columns across long time periods, and the licking response provides a means to test reaction times as short as 100 ms under a variety of experimental conditions.

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Behavior Chart

Rat # _____

DATE _____ TIME _____	5min	10min	15min	20min	25min	30min	35min	40min	45min	50min	55min	60min	65min	70min	75min
Observer _____															
GOOD BEHAVIOR		GOOD		GOOD		GOOD		GOOD		GOOD		GOOD		GOOD	
BAD BEHAVIOR		BAD		BAD		BAD		BAD		BAD		BAD		BAD	
waking		waking		waking		waking		waking		waking		waking		waking	
calm waking		calm waking		calm waking		calm waking		calm waking		calm waking		calm waking		calm waking	
sleeping		Sleeping		Sleeping		Sleeping		Sleeping		Sleeping		Sleeping		Sleeping	

Notes:

Figure 1. Example of the ethogram

A 60 minute ethogram was used for our pre-training protocol. Various behaviors typically elicited by rats under restraint were categorized as good or bad behavior to meet the needs of our experiment. The specific behavior during each 30 second interval was recorded throughout the entire training period. At the end of training, good and bad behaviors were totaled to get a percentage of good behavior.

Figure 2. Homemade wraps and hammocks

Our wrap illustrated in the upper left panel (A) was made of one piece of fabric with V-shaped arms to go around the neck, and legs to cross over the rear of the animal. The Velcro along the side folds around the animal to contain the body. The lower left panel (B) shows a picture of a rat in one of our homemade wraps. The homemade hammocks illustrated in the upper right panel (C) have holes for the rat's legs to hang through and a lace up back to snugly enclose the rat, as seen in the lower right panel (D).

Figure 3. Electrode matrix and its position over the cortex

The 64-channel electrode array was used to map field potentials on the surface of the somatosensory cortex while the rat performed the licking task. Screw electrodes were used for recording frontal and parietal EEG. Stainless steel wire was threaded across the rib cage to record EKG and inserted into the neck muscles to record EMG. Two additional screws (Ground) served as reference electrodes. Several anchor screws were placed around the skull for additional headstage support.

Figure 4. Experimental design

An illustration of the experimental set-up for licking response conditioned learning paradigm shows an animal in a hammock. The rat's head was restrained with earbars that are usually inserted into a hypodermic tube cemented onto the headstage. A reward and control whisker were inserted into two different hypodermic tubes attached to whisker deflectors for stimulation. The rat was trained to lick to receive a water reward when one whisker was stimulated (reward whisker), and not to lick after a control whisker was stimulated. The optical lickometer was placed close to the rats mouth, with an infrared beam in the path of the tongue and a fluid delivery tube. A set of 64 pre-amplifier circuits were connected to the rat's headstage to record cortical surface activity and evoked responses.

Figure 5. Progress of training (% good behavior/bad behavior)

The behavioral progress of pre-training, involved full body restraint over a 30 day period. The top graph (A) shows the percentage of good behavior to total recorded behavior, calculated from the data provided in the ethograms seen in Figure 1. The lower graph (B) shows the increase in time spent in the wrap during training. Rats were typically kept in the wrap as long as possible, unless they became stressed (excessive chattering, fluid buildup in eyes, excessive wiggling), in which case they were unwrapped and returned to their home cage. Since some animals were excluded due to excessive bad behaviors or lack of progress, this study began with 24 animals on day 1, with 5 animals remaining by day 30.

Figure 6. Recorded traces during stimulation

In this task, the conditioned sensory stimulation (whisker twitch) was presented, which either predicted a water reward (reward) or no reward (control) upon licking depending on the specific whisker that was twitched. Rats were trained to differentiate between twitching of one whisker, which was rewarded, and another which served as the control. Lick response, EEG, and EMG are shown immediately pre- and post-stimuli for both the reward and control whiskers.

Figure 7. Peri-stimulus histograms of conditioned learning

Typical lick response peri-stimulus histograms after stimulation of the reward and control whiskers shows improvement over four days. The first lick within a 2 second period post-stimulation was counted as a response. Histograms show the progress from day 1 to day 4 of conditioned learning with faster and more consistent licks only after the reward whisker was twitched.

Figure 8. Mapping of electric potentials

EEG from our 64 channel electrode array were sampled at a rate of 20kHz (filtered between 0.1 Hz and 3.2kHz and amplified by a factor of 1,000) and collected by the computer while simultaneously twitching various whiskers. Triggered responses to twitching were averaged (about 50 stimuli). A topographical map of electrode potentials was generated in order to localize individual cortical columns corresponding to different whisker barrels under the array. For example, twitching whiskers C0 and C2 (A) each produced unique maps. The electrode with a peak amplitude associated with the location of the whisker barrel underneath the array, corresponded to that particular whisker. The evoked response on the peak channel (B) can then be plotted over time to measure the amplitude and shape of the response produced by the whisker twitching under various conditions.

Figure 9. Evoked responses pre-training and 1 week after training

Evoked responses recorded throughout the lick training showed $25 \pm 8\%$ larger amplitudes one week after training commenced (black line) relative the the pre-training period (gray line) ($p < 0.05$). Two examples are shown here, corresponding to two rats, rw020708 and rw013008. The vertical line indicated the stimulus time.