

Behavioral verification of associative learning in whisker-related fear conditioning in mice

Anita Cybulska-Kłosowicz

*Laboratory of Neuroplasticity, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland,
Email: a.cybulska@nencki.gov.pl*

Fear-conditioning is one of the most widely used paradigms in attempts to unravel the processes and mechanisms underlying learning and plasticity. In most Pavlovian conditioning paradigms an auditory stimulus is used as the conditioned stimulus (CS), but conditioning to a tactile CS can also be accomplished. The whisker-to-barrel tactile system in mice offers a convenient way to investigate the brain pathways and mechanisms of learning and plasticity of the cerebral cortex. To support the claim that an animal learns during conditioning sessions and that the resulting plastic changes are associative in nature, objective measures of behavior are necessary. Multiple types of conditioned responses can develop depending on the training situation, CS and unconditioned stimulus (UCS) characteristics. These include physiological responses such as salivation, heart rate, and galvanic skin reaction, and also behavioral responses such as startle reflex potentiation or suppression of an ongoing behavior. When studying learning with the whisker system in behaving mice, stimulation of individual whiskers in a well-controlled manner may require animal restraint, which has the disadvantage of limiting the observation of potential behavioral responses. Stimulation of whiskers in a neck-restraining apparatus evokes head movements. When whisker stimulation (CS) is paired with an aversive UCS during conditioning, the number of head movements decrease in the course of the training. This reaction, called minifreezing, resembles the frequently used behavioral measure known as the freezing response. However, this is only applicable for freely moving animals. This article will review experimental evidence confirming that minifreezing is a relevant index of association formation between the neutral CS and aversive UCS.

Key words: vibrissae, barrel cortex, delay conditioning, conditioned reaction, freezing

INTRODUCTION

One of the simplest forms of learning is classical (Pavlovian) conditioning. In a typical experiment a neutral conditioned stimulus (CS) is systematically paired with an aversive or appetitive unconditioned stimulus (UCS), which elicits an unconditioned reaction (UR). The previously neutral CS thereby acquires aversive or appetitive properties, and when subsequently presented alone will itself evoke reaction (conditioned reaction, CR). This form of learning is easy to employ and execute. This is also an experimental procedure that enables studying and analyzing the process of associative learning and its effects at a behavioral and neurobiological level. In most Pavlovian conditioning experiments an auditory stimulus, i.e. a tone (CS), is paired with an innately aversive stimulus (e.g. electric shock; UCS) during training. Studies using this procedure, referred to as auditory fear conditioning, date from the fourth decade of the 20th century (e.g. Grant and Schneider 1949, Brown et al. 1951, Galambos et al. 1956). Since then auditory fear conditioning has been used extensively to study fear learning and memory (for review

see Johansen et al. 2012) and auditory cortical plasticity (for review see Weinberger 2007a, 2007b). Stimuli of other modalities, such as visual (e.g. Petro et al. 2015), odorant (Davis 2004) or taste (Davis and Riley 2010), can also be used as CSs. Fear conditioning to tactile CS can also be accomplished; the vibrissae and whisker-to-barrel tactile system in mice offers a convenient way to investigate the brain pathways and neural mechanisms of learning and memory, and is now the most popular model for studying plasticity of the brain cortex.

Each mystacial whisker is represented in layer IV of the primary somatosensory cortex (S1) by well-defined structures called barrels. Barrels in the cortex are somatotopically arranged in a pattern that is identical to the arrangement of whiskers on the muzzle (Woolsey and Van der Loos 1970). The one-to-one correspondence between whiskers and barrels is useful for investigations of cortical development, structure-function relationships, sensory processing and experience, and learning dependent plastic changes in cortical pathways. The whiskers-to-barrels system is one of the best studied sensory systems, and large amounts of neuronal data on cortical microcircuits and

synaptic connectivity, on both the cellular and molecular level, are already present.

Numerous and various experimental paradigms involving the whisker-to-barrel system have been developed, including all whiskers deprivation (Glazewski et al. 1998), single whisker deprivation (Glazewski et al. 1998, Allen et al. 2003), single whisker sparing (Fox 1992, Bender et al. 2006, Clem and Barth 2006, Benedetti et al. 2009), double whisker sparing (Diamond et al. 1994), and chessboard deprivation (Wallace and Fox 1999) and others (Jasińska et al. 2014). Moreover, several methods of sensory training in which the vibrissae system is engaged in learning have been developed, such as the gap-crossing task (Barnéoud et al. 1991, Troncoso et al. 2004), object localization tasks (e.g. O'Connor et al. 2010, Kuhlman et al. 2014), the texture discrimination task (Guic-Robles et al. 1989, Cybulska-Kłosowicz and Kossut 2001, Wu et al. 2013, Zuo et al. 2015), and the vibrotactile frequencies discrimination task (e.g. Mayrhofer et al. 2013). It is also well recognized and documented that vibrissae stimulation can be used as a conditioned stimulus (CS) in classical conditioning procedures (Siucinska and Kossut 1996, Galvez et al. 2006). Combining the particularly convenient whisker-to-barrel model with the simple and well recognized classical conditioning procedure seems to be an excellent choice to study pathways and mechanisms of associative learning processes and accompanying cortical plasticity.

Although the auditory type of fear conditioning is the most common approach in experiments and the auditory system is very well described, there were substantial controversies about the results of associative plasticity in the primary auditory cortex. These included opposing claims regarding the form of receptive field plasticity, the interpretation of its functional significance, and its underlying neural mechanisms. Many of the early studies did not include the control procedures or behavioral verifications necessary to establish that learning occurred (e.g. unpaired or random control groups, after-training trials; Papini and Bitterman 1990, Rescorla 1967, 1988a, 1988b), and only the pioneering studies of Weinberger and his colleagues used appropriate controls (e.g. Diamond and Weinberger 1986). According to Weinberger, the lack of behavioral validation of learning does not invalidate the possibility of learning-induced plastic changes; however, behavioral validation is necessary to support the claim that the observed changes are indeed associative in nature (for review see Weinberger 2007a, 2007b).

CLASSICAL FEAR CONDITIONING – FREEZING RESPONSE AS A BEHAVIORAL MEASURE

To prove and confirm that an animal actually learns during a learning session, the animal's behavior has to be

quantified using some kind of response measures. Multiple CRs might develop while the CS–UCS association is being learned, depending on the CS and UCS characteristics and the intensity, animal species, motivational system involved, etc. In aversive conditioning CRs include physiological responses, such as changes in blood pressure, heart rate, salivation, galvanic skin response, and respiration, and also behavioral responses such as facilitation of the startle reflex or suppression of an ongoing behavior. Different types of behavioral responses that develop as the result of aversive, fear conditioning have been described and explained in the model of Bolles and Fanselow (1980, 1982). According to this model, different defensive responses are mediated by two motivational systems: one related to fear and the other related to pain. The pain-motivational system is activated by nociceptive stimuli and serves to protect the animal from bodily injury through an active form of behavior, i.e. the startle reflex, such as jumping and running. The fear-motivational system is activated by a stimulus that might pose danger to the animal. A pattern of inhibitory responses, i.e. the freezing response, is the main defensive behavior triggered by this system. The startle reflex and freezing are universal fear responses seen in many animal species, and can be easily conditioned to light, tones and contexts using fear-conditioning paradigms, where foot shock is used as the UCS (e.g. Fanselow 1980, Fanselow and Poulos 2005).

The freezing response is a good behavioral measure in freely moving animals. The onset of the CS results in the cessation of all ongoing behavior in a fear conditioned mouse or rat. Animals typically adopt a characteristic crouching or “freezing” posture (Blanchard and Blanchard 1969). It has been argued that freezing behavior is one of the primary responses, that it is innate and unconditioned, and that it can be classified as a preparatory reflex of rodents to current or anticipated danger; it functions as an attempt to escape detection by a predator or potential threats (Blanchard and Blanchard 1969, Bolles and Fanselow 1980, Fanselow and Lester 1988, Rosen and Schulkin 1998). When saying that freezing is a Pavlovian response, it means that is not controlled by its effects on the environment, i.e. it is not an operant response (Fanselow 2000). In operant conditioning an animal controls shock delivery by adopting the freezing posture, while in classical fear conditioning the animal freezes in response to the CS that announces the shock. In other words, what controls the conditioned freezing response is the relationship between the CS and UCS stimuli. Direct measures of freezing to CSs such as tones and lights began in Robert Bolles' laboratory in the late 1970s (e.g. Fanselow and Bolles 1979, Collier and Bolles 1980), and thereafter the freezing response is considered to be a suitable index of learning a Pavlovian stimulus–shock relationship. It is a tense and attentive immobility, refraining all movements except those associated with

respiration and eye movements (Misslin 2003). Differences in the level of freezing during CS presentations are used to infer alterations in the acquisition or expression of CS fear. Many reports suggest that fear conditioning is a direct function of the intensity of the UCS. For example, Morris and Bouton (2006) observed that the point in conditioning training at which freezing emerged and the asymptotic amount of freezing was directly related to the intensity of the UCS (footshock). Other studies indicate that performance in conditioning training is an inverted-U function of footshock intensity (Leaton and Borszcz 1985, Witnauer and Miller 2013), and intermediate footshock intensities appear to be the most useful for eliciting a conditioned freezing response to the CS (Baldi et al. 2004). With an UCS of higher intensity a conditioned response to context may develop, and further increasing of footshock intensity may cause the generalization phenomenon to occur (Baldi et al. 2004), where the animal freezes when no CS is presented and even outside the conditioning context (Fanselow 1980). On the other hand, repeated administration of intensive stimuli (UCS) may result in progressive amplification of response of the animal even to harmless stimuli (CS), a process called sensitization. The footshock might sensitize the animal when administered alone, but also if presented together with a CS during conditioning. Consequently, the freezing response to the CS following conditioning might be determined not only by associative, but also by non-associative components. It has been postulated that the intensity of freezing as a function of footshock intensity is primarily determined by the non-associative sensitization component, whereas the associative component is more or less categorical. Thus, when the sensitization component is calculated out, no differences in the intensity of response is observed in mice conditioned with different intensities (Kamprath and Wotjak 2004). Results from the above-mentioned studies and the study of Sigmundi and colleagues (1980) indicate that a number of variables in addition to/together with shock intensity may influence the level of freezing. They demonstrated that the degree of conditioned freezing might depend on the CS modality: when a white noise was the CS conditioned freezing increased with shock intensity, and when a localized light was the CS freezing did not vary with shock intensity (Sigmundi et al. 1980).

Changes in fearful behaviors observed after pairing the CS and UCS are presumed to be due to the formation of an association between these stimuli. However, due to the aversive nature of the UCS and the novelty of the CS and UCS, fearful behaviors can emerge to the CS despite the absence of an association. This stimulus-elicited behavior can be similar to the anticipated conditioned reaction, but can only be accounted for by non-associative mechanisms. Another possibility is that the observed fearful behavior is not an UR but a delayed UR (Fanselow 2000). To determine

that a behavioral change is due to the formation of an association and not merely by exposure to the stimuli, unpaired (Papini and Bitterman 1990) or random control groups (Rescorla 1967) are introduced in which mice are exposed to both the CS and UCS, but are unable to form an association between them. If freezing is a CR it should be CS specific. If freezing is an UR it should be time locked to the UCS presentation; substantial delays or changes in the temporal relationship between shock and testing should reduce freezing.

Classical conditioning paradigms involving whiskers

The majority of studies combining the whisker-to-barrel model in rodents with the fear conditioning procedure investigated the brain pathways and neural mechanisms underlying learning-dependent associative plasticity in the somatosensory system and memory processes. To support the claim that the observed plastic changes are associative in nature, objective measures of behavior are necessary.

One of the most convenient paradigms for measuring a behavioral conditioned response is eyeblink conditioning, since the CR in this paradigm is robust, reliable, and discrete (Thompson 2005). This associative learning paradigm was first developed for use in human participants in the 1920s (Cason 1922), and then the need for an animal model led to the development of the rabbit eyeblink and nictating membrane paradigms (Gormezano et al. 1962, Schneiderman et al. 1962); later on the paradigm was also applied to frogs, turtles, mice, rats, ferrets, sheep, dogs, monkeys, and cats. The procedure involves the paired presentation of a CS, typically a tone or light, with an UCS that reliably elicits eyelid closure, such as an air puff or brief electrical stimulation near the eye. The eyeblink reflex in the rabbit is a coordinated response involving simultaneous and correlated external eyelid closure, eyeball retraction, and resulting passive extension of the nictitating membrane (e.g. Gormezano et al. 1962, McCormick et al. 1982). “Eyeblink” is often used synonymously for eyelid and nictitating membrane movement because the premotor neural circuitry underlying the respective CRs is the same. However, the motor nuclei that generate eyelid and nictitating membrane movement are distinct (reviewed by Freeman and Steinmetz 2011). Moreover, different (in kinematics and neural control) types of blinks are elicited by different stimuli (Gruart et al. 1995, Trigo et al. 1999, reviewed by Freeman and Steinmetz 2011). Eyeblink conditioning, in which whisker stimulation serves as the CS (whisker-trace-eyeblink), was invented and used by Galvez and colleagues to examine experience-induced neuronal plasticity in the somatosensory whisker pathway, at first in rabbits (Galvez et al. 2006) and then also in mice (Galvez et al. 2009, 2011, Chau et al. 2014). The piezoelectric stimulator

allows for stimulation of whiskers in a freely moving mouse while electrodes delivering periorbital shock (UCS) and an optic sensor monitoring eyeblinks are attached to a head bolt and affixed to the animal's skull, allowing for CRs to be measured reliably. The whisker stimulator in this procedure offers a good tool for time controlled stimulation. However, it is not really useful if it is required to determine precisely which whisker is being deflected. Stimulation of specific whiskers can be achieved only by trimming the undesired whiskers to prevent contact with the stimulator comb. It is therefore not useful when the procedure consists of several sessions of stimulation in which the animal is returned to the home cage between training sessions and is supposed to spend that time in standard, control conditions. Trimming whiskers has been shown to induce rapid plastic changes in the size of the receptive fields for the trimmed whiskers (Diamond et al. 1993, 1994, Armstrong-James et al. 1994).

Another work in which classical conditioning with vibrissae stimulation (CS) was studied in freely moving mice was the study by Gdalyahu and colleagues (2012). Passive whisker stimulation was achieved by attaching small pieces of metal wire to whiskers and placing the animal in an electromagnetic field, where free exploration was possible. Whisker stimulation (CS) was paired with a foot shock and freezing behavior was considered as a CR. One of the control groups of mice received the same stimuli but explicitly unpaired and another control group, trained with stimulation only, received the paired procedure but no US. In contrast with the whisker-trace-eyeblink described above, this paradigm allows for a precise stimulation of the chosen, specific whisker. The authors examined if the fear response could be evoked by stimulation of either an adjacent or distant untrained whisker, and found no generalization to a distant whisker but did find generalization to an adjacent untrained whisker. This result seems to point to a general characteristic of learning with whiskers in rodents, since it has been previously shown that rats generalize gap-crossing learning to an adjacent but not to a remote whisker (Harris et al. 1999). Another dimension of generalization was also examined in the study, and the results revealed that that fear response generalized to stimulus frequencies different from that used during training. The paradigm used by Gdalyahu and others (2012) has the disadvantage of requiring that metal pieces be glued to the whiskers. Rodents incessantly try to remove any strange object from their whiskers. This would introduce an uncontrolled, additional stimulation to those whiskers and often results in the mouse either chewing the wire off or physically removing their whisker entirely. Therefore, the mouse needs to wear a special collar in that task to avoid an increased and uncontrollable stimulation or even whisker removal.

Whisker stimulation in the classical conditioning paradigm was also applied to neonatal rat pups (Landers

and Sullivan 1999, Sullivan et al. 2003). Neonatal rats were placed in Petri dishes and manual stimulation of vibrissae was paired with the UCS – heat from a warm air stream or electric shock, delivered to the trunk. The behavior of pups was recorded prior to stimulation as a baseline and during training using a motor activity scale designed by Hall (Hall 1979). This scale assigns a score for the number of major body parts or regions that moved for at least 2 s. Specific movements such as crawling, head turning toward and away from the stimulation, and mouthing were counted (Sullivan et al. 2003). The motor activity scale measures changes in general motor activity as a response of motorically immature rat pups to stimulus presentation (Hall 1979). A behavioral evaluation protocol for learning was applied and acquisition curves were used to assess learning. Associative pairing of a whisker stimulation (CS) and unconditioned stimulus produced a conditioned behavioral activation response (generalized increase in behavioral activity) to the whisker CS alone (Landers and Sullivan 1999).

When studying learning with the whisker system in behaving mice, stimulation of individual whiskers in a well-controlled manner often requires immobilization of the animal. This can be achieved with the use of head holding elements fixed to the head of the animal (Rosselet et al. 2011, Wróbel et al. 1995, Musial et al. 1998, Jakuczun et al. 2005) or special neck-restraining apparatus (Siucinska and Kossut 1996). In these studies whiskers were stimulated with piezoelectric or mechanical (low tone audio-speakers) stimulators (Wróbel et al. 1995, Musial et al. 1998, Jakuczun et al. 2005) or manually (Rosselet et al. 2011, Siucinska and Kossut 1996), and a mild electric shock was applied to the skin of the ear or to the tail as the UCS. Conditioned response was monitored by measuring cardiac responses with an electrocardiogram (Siucinska and Kossut 1996, Cybulska-Kłosowicz and Kossut 2006, Rosselet et al. 2011). Heart rate deceleration during application of the CS was observed in these studies. This bradycardia resulted from associative learning as it was not seen in the group of pseudoconditioned mice, for which the occurrences of whisker deflections and shocks were uncorrelated during training. Although the physiological conditioned response was described, behavioral measures of the conditioned reaction were not described in these studies (Rosselet et al. 2011, Wróbel et al. 1995, Musial et al. 1998, Jakuczun et al. 2005, reviewed in Kublik 2004 and the early studies of Kossut group). The necessity of restraint during conditioning paradigms involving whiskers ensures access for well-controlled whisker stimulation. However, the disadvantage of restraint is that only limited behavioral reactions can be observed in restrained animals. Only a short mention on the changes in behavior manifested in a rigid posture in conditioned mice was provided in the study of Siucinska and Kossut (1996).

Minifreezing

Behavioral conditioned reaction in classical conditioning is an indication of association formation. Therefore when studying the mechanism of associative learning in this experimental paradigm, it is essential to measure changes in behavior. The short note on the behavioral observations in the study of Siucinska and Kossut (1996) was a starting point for more detailed descriptions and assessments of conditioned reaction in subsequent studies by our group. During whisker-related delay conditioning that training, whiskers on one side of the snout (always the same in all animals) were stroked (CS) with a fine hand-held brush. The CS lasted 9 s. During the last second, an aversive UCS (a mild, non-painful electric shock given to the tail) was delivered and co-terminated with the CS. After a 6-s interval the trial was repeated and this routine continued for 10 min. The training sessions were repeated once a day for three days. Prior to behavioral training, the mice were placed in a neck restraint for 10 min a day, 5 days a week, for 2–3 weeks to habituate them to limited mobility. The neck-restraint allows for head movements in some limited range. In the first sessions of habituation mice moved vigorously in the apparatus, and after several sessions they stayed quiet, yet not motionless. Prior to conditioning but after habituation, the stimulation of whiskers in the restrained mice evoked head movements towards the stimulus, and this most probably constituted a part of the orienting response. During conditioning, mice reacted to vibrissal stimulation during the first trials of the first training session by moving their heads in response to the stimulation, but during the following trials the frequency of head movements decreased (Fig. 1A). Trials during which a mouse reacted by head movement in response to stimulation of vibrissae (CS) were counted. The results were presented as a percentage of trials during which head movements were observed in subsequent minutes of training. Head movements occurring during the application of the tail shock and/or during the inter-trial interval in the conditioning training were not included. The natural, unconditioned reaction to tail shock in the restraining apparatus is the cessation of head movements. The behavior of mice was also analyzed when the tail shock (UCS) was applied alone in the same time schedule as during CS+UCS training. The number of head movements, counted during the same time windows as during standard CS+UCS training, was very low starting from the very first trial of the stimulation. The behavior of mice during the training was video-recorded for off-line analysis.

During conditioning the frequency of head turning in response to the CS was found to decrease in the course of conditioning to a level comparable to that observed during application of UCS only (Cybulska-Klosowicz et al. 2009). A significant reduction in head movements was observed as early as the third trial of the first session. The number of

head movements remained low and did not differ between the first and final trials of the second and the third training sessions (Cybulska-Klosowicz et al. 2013b; Fig. 1A). Rescorla has indicated the dangers of relying only on data obtained during training to infer the strength of learning, and he emphasized the need for appropriate post-training assessments of behavior (Rescorla 1988a, 1988b). Therefore, the frequency of head movements was measured by presentation of the CS during extinction trials conducted 24 hours after conditioning, and the results revealed that the frequency of conditioned responses remained low, at a level comparable with the third training session (Cybulska-Klosowicz et al. 2009; Fig. 1A). The behavior of conditioned mice contrasted with the behavior of mice that underwent pseudoconditioning or whisker stimulation only (CS only), where the head movements are frequent and the frequency of head turning did not significantly change in the course of the session (Radwanska et al. 2010).

The observed inhibitory response, i.e. reduced frequency of head movements in mice aversively conditioned in the restraining apparatus, was named “minifreezing”. Significant differences in head-turning behavior between training sessions and also between conditioned animals versus pseudoconditioned or those presented with CS only (Cybulska-Klosowicz et al. 2009) were considered as evidence of the developing association between the neutral CS and the unpleasant UCS. The previously neutral CS acquired aversive properties, and when subsequently presented alone itself evoked an aversive emotional reaction. A behavioral conditioned minifreezing response corresponds well with previously reported conditioned physiological response – heart rate deceleration during application of the CS (Siucinska and Kossut 1996, Cybulska-Klosowicz and Kossut 2006). This kind of association between freezing and pattern changes in cardiovascular functioning, particularly the decrease in heart rate, has also been documented in other experimental paradigms (Yoshimoto et al. 2010). The circumstances under which CS paired with an electric shock is presented influence the direction of the heart rate response. In unrestrained rats, increased heart rate accompanies the conditioned stimulus, whereas decreased heart rate accompanies the conditioned stimulus in restrained rats (Martin and Fitzgerald 1980).

During appetitive whiskers-related conditioning, where the schedule of the training was the same as in the aversive conditioning but sweetened water was used as the UCS, mice reacted vigorously to stimulation of the vibrissae during the whole of the first session. During the third session a high but significantly decreased number of head movements and an increase of head turns toward the syringe containing sweetened water was reported, which means that mice learned to actively seek the reward (Cybulska-Klosowicz and Kossut 2006, Cybulska-Klosowicz et al. 2009). The head movements in response to whisker

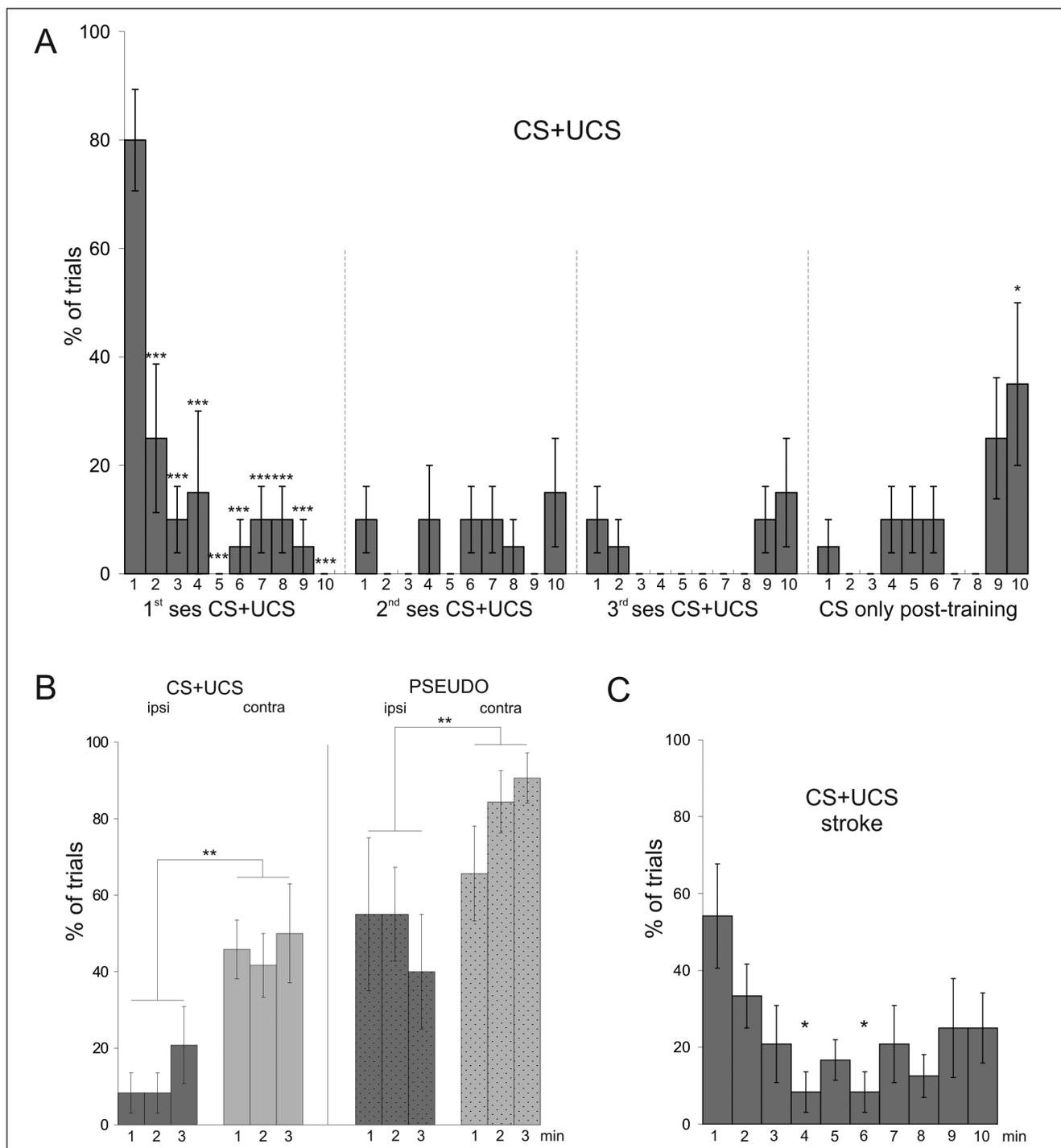


Fig. 1. (A) Assessment of behavior during all three sessions of CS+UCS conditioning and 24 hours after conditioning. Percentage of trials in subsequent minutes of the training during which head turning behavior was observed. A significant reduction in head movements was observed during the first session of conditioning (1st ses CS+UCS; N=5). The number of head movements remained low during the second (2nd ses CS+UCS; N=5) and the third (3rd ses CS+UCS; N=5) conditioning training sessions. Post-training assessment of behavior (CS only post-training; N=5) revealed extinction of the conditioned response; the head movement level increased in the 10th minute of stimulation. N - number of animals; mean \pm SE; * p<0.05, *** p<0.001, in comparison with the 1st minute of the particular session. One-Way ANOVA with Tukey-Kramer post-test. (B) Sensory stimulus specificity. CS+UCS - a group of mice that underwent standard 3 day long conditioning training; PSEUDO - a group of mice pseudoconditioned in 3 day long training. One day after completion of training, whiskers were stimulated for 3 minutes on the same side of the snout (ipsi) or on the side contralateral (contra) to the side that was stimulated in training. For statistical comparisons, data from the first 3 minutes of the training were pooled together. mean \pm SE; ** p<0.01, t-test. (C) Conditioned reaction development after a stroke in the barrel field. CS+UCS - conditioning, N=6; mean \pm SE; * p<0.05, One-Way ANOVA with Tukey-Kramer post-test.

stimulation in naive mice and at the beginning of the training most probably constituted a part of the orienting response. The change in head movement behavior in the course of the training was specific to UCS valence. Therefore the specificity of the CR was related to the value that the CS gained during conditioning.

What controls the conditioned response is the relationship between the specific stimuli in the experimental situation. The minifreezing conditioned response in our paradigm is controlled by the relationship between vibrissae stimulation on a particular side of the snout and electrical shock. Additional experiment was designed in order to ensure and confirm that minifreezing is indeed the manifestation of acquiring a predictive value by the specific CS. Potential fear response evoked by stimulation of distant, untrained whiskers on the contralateral side of the snout would be an indication of generalization, which occurs when the new stimulus shares common features with the stimulus used in the original learning (Ramos 2014). One group of mice underwent the standard 3 day long conditioning training (CS+UCS; CS – stimulation of row B of whiskers on one side of the snout; UCS – tail shock) and the second one a 3 day long pseudoconditioning (PSEUDO). One day after the end of the training, the CS only was presented in the same schedule as during the training. However, in two groups of mice (conditioned and pseudoconditioned) row B of whiskers on the same side of the snout as in the training (ipsi) were stimulated and in the other two groups (conditioned and pseudoconditioned) on the side contralateral to the one stimulated during training (contra). The stimulation session lasted only 3 minutes, because extinction could be expected after longer presentation of the CS when not paired with the UCS, as was previously observed in the 9th and 10th minutes of post-training CS only stimulation (Fig. 1B; Cybulska-Klosowicz et al. 2009).

The decrease of the frequency of head movements in mice stimulated on the same side of the snout as during the training (Fig. 3, CS+UCS, ipsi) were comparable to the results observed during the third training session (Cybulska-Klosowicz et al. 2009, Jasinska et al. 2010, Cybulska-Klosowicz et al. 2013a), both for conditioning (5–10%) and pseudoconditioning (28–68%). However, when the whiskers on the “untrained” side of the snout were stimulated (contra), head turning behavior frequency was significantly higher. In the case of conditioned mice stimulated on the contralateral side (CS+UCS, contra), the frequency of head movements was similar to the number of head movements in pseudoconditioned mice, stimulated on the same side as in the training (PSEUDO, ipsi). Mice that were pseudoconditioned and stimulated on the contralateral side (PSEUDO, contra) moved their heads with a very high frequency one day after the training, reacting to the vibrissae stimulation in ~80% of

trials, which resembles the behavior of mice stimulated for the first time (72–93%, Radwanska et al. 2010, Cybulska-Klosowicz et al. 2009, Cybulska-Klosowicz et al. 2013b; Fig. 1A – 1st minute of the training CS+UCS). These results confirmed that the association between the UCS and CS was formed. Only the specific CS and the predicted UCS were recognized, and no significant generalization occurred. Stimulation of whiskers on the side contralateral to the one stimulated during conditioning probably warned about the possibility of UCS appearance, but was overly different from the CS and did not enable it to be predicted precisely. Taking into account the aforementioned results on generalization by Gdalyahu and others (2012) showing that mice generalize the learning to an adjacent whisker but not to a remote whisker, it would be interesting to introduce another control for generalization, which would involve stimulation of the row of whiskers on the same side of the snout as during conditioning but adjacent or distant from the trained one.

Acquiring the association during conditioning – the role of the sensory cortex

Associative processes modify the cortical representation of the conditioned stimulus. Engaging the whisker-to-barrel system in mice through fear conditioning involves a widely distributed network of neural changes and results in plastic changes in the barrel cortex. Pavlovian conditioning takes place by the convergence of pathways transmitting the conditioned and unconditioned stimuli, and the sensory areas that process the CS and UCS are involved in these circuits (Johansen et al. 2012). Therefore we have undertaken an attempt to recognize the role of the barrel cortex in the acquisition of conditioning at the behavioral level.

As previously described, during application of the CS in the first training session of aversive conditioning, head movements in mice were gradually rarer and significantly reduced as the session progressed, and finally remained at a very low level (Fig. 1A). When mice were conditioned after a stroke in the barrel field, the number of head movements during whisker stimulation in the first training session decreased significantly in the 4th–6th trials, but afterwards increased again (unpublished results, Fig. 1C). Finally, there was no significant difference in head movement frequency between the first and final trials of the session. Our recent results showed that normal functioning of inhibitory circuits is critical for cortical plastic change of the whisker representation and for maintenance of the conditioned reaction. It has been shown previously that whisker-related conditioning results in an increase in the GABA level and other markers related to the GABA-ergic pathway in the barrel cortex (Siucinska et al. 1999, Gierdalski et al. 2001, Siucinska 2006, Tokarski et al. 2007,

Jasinska et al. 2010, Urban-Ciecko et al. 2010, Liguz-Leczna et al. 2014). After local cortical injection of a glutamic acid decarboxylase inhibitor – 3-mercaptopropionic acid or gabazine, an antagonist of GABAA receptors in the barrel cortex (Posluszny et al. 2015) – in the first conditioning session the learning curves were similar in the control and experimental groups. However, the reduction in the number of head movements was not a stable effect in the experimental group of mice, and a rebound (increase in head turning behavior) between the second and third conditioning session were observed in these animals. These results indicate that lesion of the barrel cortex or its dysfunction interferes with acquisition of a stable level of whisker-related learning.

The primary somatosensory cortex plays an essential role in acquisition of trace eye-blink conditioning with a tactile CS, and is also required for retention of trace-association (Galvez et al. 2007). However, the training described above is a delay conditioning, and it has been shown that lesions of the barrel cortex had no effect on this type of eye-blink conditioning (Galvez et al. 2006, 2007). Circuitry for a simple CS–CR association can be completely subcortical, so that it survives cortical lesions. Hutson and Masterton (1986) have shown that lesion of the barrel cortex does not prevent conditioning of detection and discrimination performance of tactile frequency. In that study the UCS was presented simultaneously with the CS, and thus was delay conditioning rather than trace conditioning. The difference between trace and delay conditioning is that in delay conditioning the CS and UCS are presented simultaneously and the emergence of the CS overlaps with or is immediately followed by the UCS, while in trace conditioning presentation of the CS and US is separated in time by an inter-stimulus interval. A possible interpretation, as proposed by Feldmeyer and colleagues (2013), is that the successful association of the CS and UCS in delay paradigms (Hutson and Masterton 1986, Galvez et al. 2007) can be achieved by their simultaneous occurrence and do not require the involvement of memory functions, contrary to the trace paradigm (Galvez et al. 2007), where the time interval between the CS and US requires the formation of a temporal relationship between the two stimuli. Actually, there are studies indicating that the key brain structures for trace conditioning are the cerebellum, hippocampus, and neocortex (Weiss and Disterhoft 2011). It differs from delay conditioning, where the critical structures are the cerebellum and brainstem (Clark et al. 1984, Mauk and Thompson 1987), while the cortex is not essential. However, the sensory cortex may modulate or support subcortical pathways that subservise delay conditioning, and also in parallel with these subcortical circuits can store CS–UCS information long term (Halverson et al. 2009). Information stored in the cortex can affect behavior by control over subcortical systems (Weinberger 2007b). It has been shown in delay whisker-related conditioning that activation of the

barrel cortex, thalamic sensory nuclei (ventroposteromedial thalamic nucleus and posterior thalamic nucleus), nucleus accumbens core, and posterior parietal cortex decreases with the duration of the training (final training session in comparison with the first session) (Cybulska-Kłosowicz and Kossut 2006, Cybulska-Kłosowicz et al. 2009). At the same time, correlations of activity between these brain regions increase significantly. Strengthened correlations between structures of the thalamocortical loop together with reduced metabolic activity in the final phase of conditioning enhance the efficiency of sensory processing (Cybulska-Kłosowicz et al. 2013a).

SUMMARY AND CONCLUSION

The review provides a summary of behavioral evidence of learning in studies in examining learning-dependent plasticity using classical conditioning paradigms with vibrissae stimulation as a conditioned stimulus (CS). Unfortunately, when working with the whisker system, unless one is utilizing an anesthetized or immobilized animal, the precise stimulation of individual whiskers in the well-controlled manner required by most learning paradigms is difficult. One of the most convenient paradigms for this kind of studies is eyeblink conditioning, namely the whisker–trace eyeblink, which allows for a very reliable measure of CRs in a freely moving mouse. In this method the stimulator and sensor monitoring CRs are affixed to the animals' skull, which might appear to be a disadvantage in some experimental procedures. In experiments where whisker-related conditioning is carried out in a neck-restraining apparatus, the reliable method to demonstrate development of the conditioned reaction is evaluating head-turning behavior. This refers only to experiments that do not require stable or fixed head position. In experimental situations that require a stable head-fixed models, head-turning behavior is blocked and cannot be used to assess the conditioned reaction. In the neck restraining apparatus the animal is able to move its head in some range, and this gives the opportunity to assess the conditioned behavioral reaction. The reduction in head movements observed in mice aversively conditioned in the neck-restraining apparatus is similar to the freezing behavior observed as a result of fear conditioning, where foot shock is used as the UCS. This conditioned reaction, called “minifreezing”, proved to be a relevant indicator of association formation between the neutral CS and the aversive UCS, and can be used for verification of associative learning in whisker-related classical conditioning training. In the experiments conducted so far the observer has made an arbitrary decision whether or not the animal moved its head; any data collected by a human observer are necessarily subjective. Automatic, observer–decision free and computer-based analysis, where the criteria are likely to be more exact

than those of the observer-scoring method, might deliver more precise and detailed results.

The involvement of the barrel cortex in learning process in these vibrissae-related conditioning paradigms is discussed in the review. Lesion or dysfunction of the barrel cortex interferes with learning acquisition in delay conditioning, where the behavioral measure of learning was head-turning behavior.

REFERENCES

- Allen CB, Celikel T, Feldman DE (2003) Long-term depression induced by sensory deprivation during cortical map plasticity in vivo. *Nat Neurosci* 6: 291–299.
- Armstrong-James M, Diamond ME, Ebner FF (1994) An innocuous bias in whisker use in adult rats modifies receptive fields of barrel cortex neurons. *J Neurosci* 14: 6978–6991.
- Baldi E, Lorenzini CA, Bucherelli C (2004) Footshock intensity and generalization in contextual and auditory-cued fear conditioning in the rat. *Neurobiol Learn Mem* 81: 162–166.
- Barnéoud P, Gyger M, Andrés F, van der Loos H (1991) Vibrissa-related behavior in mice: transient effect of ablation of the barrel cortex. *Behav Brain Res* 44(1): 87–99.
- Bender VA, Bender KJ, Brasier DJ, Feldman DE (2006) Two Coincidence Detectors for Spike Timing-Dependent Plasticity in Somatosensory Cortex. *J Neurosci* 26(16): 4166–4177.
- Benedetti BL, Glazewski S, Barth AL (2009) Reliable and precise neuronal firing during sensory plasticity in superficial layers of primary somatosensory cortex. *J Neurosci* 29: 11817–11827.
- Blanchard RJ, Blanchard DC (1969) Passive and active reactions to fear-eliciting stimuli. *J Comp Physiol Psychol* 68(1): 129–135.
- Bolles RC, Fanselow MS (1980) A perceptual-defensive-recuperative model of fear and pain. *Behav Brain Sci* 3: 291–301.
- Bolles RC, Fanselow MS (1982) Endorphins and behavior. *Annu Rev Psychol* 33: 87–101.
- Brown JS, Kalish HI, Farber IE (1951) Conditioned fear as revealed by magnitude of startle response to an auditory stimulus. *J Exp Psychol* 41: 317–332.
- Cason H (1922) The conditioned eyelid reaction. *J Exp Psychol* 5: 153–196.
- Chau LS, Prakapenka AV, Zendeli L, Davis AS, Galvez R (2014) Training-dependent associative learning induced neocortical structural plasticity: a trace eyeblink conditioning analysis. *PLoS One* 9(4): e95317.
- Clark GA, McCormick DA, Lavond DG, Thompson RF (1984) Effects of lesions of cerebellar nuclei on conditioned behavioral and hippocampal neuronal responses. *Brain Res* 291(1): 125–136.
- Clem RL, Barth A (2006) Pathway-specific trafficking of native AMPARs by in vivo experience. *Neuron* 49: 663–670.
- Collier AC, Bolles RC (1980) The ontogenesis of defensive reactions to shock in preweanling rats. *Dev Psychobiol* 13(2): 141–150.
- Cybulska-Klosowicz A, Brzezicka A, Zakrzewska R, Kossut M (2013a) Correlated activation of the thalamocortical network in a simple learning paradigm. *Behav Brain Res* 252: 293–301.
- Cybulska-Klosowicz A, Kossut M (2001) Mice can learn roughness discrimination with vibrissae in a jump stand apparatus. *Acta Neurobiol Exp (Wars)* 61(1): 73–76.
- Cybulska-Klosowicz A, Kossut M (2006) Early-phase of learning enhances communication between brain hemispheres. *Eur J Neurosci* 24(5): 1470–1476.
- Cybulska-Klosowicz A, Posluszny A, Nowak K, Siucinska E, Kossut M, Liguz-Leczna M (2013b) Interneurons containing somatostatin are affected by learning-induced cortical plasticity. *Neuroscience* 254: 18–25.
- Cybulska-Klosowicz A, Zakrzewska R, Kossut M (2009) Brain activation patterns during classical conditioning with appetitive or aversive UCS. *Behav Brain Res* 204(1): 102–111.
- Davis CM, Riley AL (2010) Conditioned taste aversion learning: implications for animal models of drug abuse. *Ann N Y Acad Sci* 1187: 247–275.
- Davis RL (2004) Olfactory learning. *Neuron* 44(1): 31–48.
- Diamond DM, Weinberger NM (1986) Classical conditioning rapidly induces specific changes in frequency receptive fields of single neurons in secondary and ventral ectosylvian auditory cortical fields. *Brain Res* 372(2): 357–360.
- Diamond ME, Armstrong-James M, Ebner FF (1993) Experience-dependent plasticity in adult rat barrel cortex. *Proc Natl Acad Sci U S A* 90: 2082–2086.
- Diamond ME, Huang W, Ebner FF (1994) Laminar comparison of somatosensory cortical plasticity. *Science* 265: 1885–1888.
- Fanselow MS (1980) Conditioned and unconditional components of post-shock freezing. *Pavlov J Biol Sci* 15: 177–182.
- Fanselow MS (2000) Contextual fear, gestalt memories, and the hippocampus. *Behavioural Brain Research* 110: 73–81.
- Fanselow MS, Bolles RC (1979) Naloxone and shock-eliciting freezing in the rat. *J Comp Physiol Psychol* 93: 736–744.
- Fanselow MS, Lester LS (1988) A functional behavioristic approach to aversively motivated behavior: predatory imminence as a determinant of the topography of defensive behavior. In: *Evolution and learning* (Bolles RC, Beecher MD, Eds.), Hillsdale, New York, USA. p. 185.
- Fanselow MS, Poulos AM (2005) The neuroscience of mammalian associative learning. *Annu Rev Psychol* 56: 207–234.
- Feldmeyer D, Brecht M, Helmchen F, Petersen CC, Poulet JF, Staiger JF, Luhmann HJ, Schwarz C (2013) Barrel cortex function. *Prog Neurobiol* 103: 3–27.
- Fox K, Daw N, Sato H, Czepita D (1992) The Effect of Visual Experience on Development of NMDA Receptor Synaptic Transmission in Kitten Visual Cortex. *J Neurosci* 12(7): 2672–2684.
- Freeman JH, Steinmetz AB (2011) Neural circuitry and plasticity mechanisms underlying delay eyeblink conditioning. *Learn Mem* 18: 666–677.
- Galambos R, Sheatz G, Vernier VG (1956) Electrophysiological correlates of a conditioned response in cats. *Science* 123: 376–377.
- Galvez R, Cua S, Disterhoft JF (2011) Age-related deficits in a forebrain-dependent task, trace-eyeblink conditioning. *Neurobiol Aging* 32(10): 1915–1922.
- Galvez R, Weible AP, Disterhoft JF (2007) Cortical barrel lesions impair whisker-CS trace eyeblink conditioning. *Learn Mem* 14(1): 94–100.
- Galvez R, Weiss C, Cua S, Disterhoft JF (2009) A novel method for precisely timed stimulation of mouse whiskers in a freely moving preparation: Application for delivery of the conditioned stimulus in trace eyeblink conditioning. *J Neurosci Methods* 177: 434–439.
- Galvez R, Weiss C, Weible AP, Disterhoft JF (2006) Vibrissa-signaled eyeblink conditioning induces somatosensory cortical plasticity. *J Neurosci* 26(22): 6062–6068.
- Gdalyahu A, Tring E, Polack PO, Gruver R, Golshani P, Fanselow MS, Silva AJ, Trachtenberg JT (2012) Associative fear learning enhances sparse network coding in primary sensory cortex. *Neuron* 75(1): 121–132.
- Gierdalski M, Jablonska B, Siucinska E, Lech M, Skibinska A, Kossut M (2001) Rapid regulation of GAD67 mRNA and protein level in cortical neurons after sensory learning. *Cereb Cortex* 11(9): 806–815.
- Glazewski S, McKenna M, Mark Jacquin M and Fox K (1998) Experience-dependent depression of vibrissae responses in adolescent rat barrel cortex. *Eur J Neurosci* 10(6): 2107–2116.
- Gormezano I, Schneiderman N, Deaux EG, Fuentes I (1962) Nictitating membrane: classical conditioning and extinction in the albino rabbit. *Science* 138: 33–34.
- Grant DA, Schneider DE (1949) Intensity of the Conditioned Stimulus and Strength of Conditioning: II. The Conditioned Galvanic Skin Response to an Auditory Stimulus. *Journal of Experimental Psychology* 39(1): 35.

- Gruart A, Blazquez P, Delgado-García JM (1995) Kinematics of spontaneous, reflex, and conditioned eyelid movements in the alert cat. *J Neurophysiol* 74: 226–248.
- Guic-Robles E, Valdivieso C, Guajardo G (1989) Rats can learn a roughness discrimination using only their vibrissal system. *Behav Brain Res* 31(3): 285–289.
- Hall WG (1979) Feeding and behavioral activation in infant rats. *Science* 205: 206–209.
- Halverson HE, Hubbard EM, Freeman JH (2009) Stimulation of the lateral geniculate, superior colliculus, or visual cortex is sufficient for eyeblink conditioning in rats. *Learn Mem* 16(5): 300–307.
- Harris JA, Petersen RS, Diamond ME (1999) Distribution of tactile learning and its neural basis. *Proc Natl Acad Sci U S A* 96: 7587–7591.
- Hutson KA, Masterton RB (1986) The sensory contribution of a single vibrissa's cortical barrel. *J Neurophysiol* 56(4): 1196–1223.
- Jakuczyn W, Kublik E, Wojcik D, Wróbel A (2005) Local classifiers for evoked potentials recorded from behaving rats. *Acta Neurobiol Exp (Wars)* 65: 425–434.
- Jasinska M, Grzegorzczak A, Jasek E, Litwin JA, Kossut M, Barbacka-Surowiak G, Pyza E (2014) Daily rhythm of synapse turnover in mouse somatosensory cortex. *Acta Neurobiol Exp (Wars)* 74: 104–110.
- Jasinska M, Siucinska E, Cybulska-Kłosowicz A, Pyza E, Furness DN, Kossut M, Glazewski S (2010) Rapid, learning-induced inhibitory synaptogenesis in murine barrel field. *J Neurosci* 30(3): 1176–1184.
- Johansen JP, Wolff SB, Lüthi A, LeDoux JE (2012) Controlling the elements: an optogenetic approach to understanding the neural circuits of fear. *Biol Psychiatry* 71(12): 1053–1060.
- Kamprath K, Wotjak CT (2004) Nonassociative learning processes determine expression and extinction of conditioned fear in mice. *Learn Mem* 11: 770–786.
- Kublik E (2004) Contextual impact on sensory processing at the barrel cortex of awake rat. *Acta Neurobiol Exp (Wars)* 64(2): 229–238.
- Kuhlman SJ, O'Connor DH, Fox K, Svoboda K (2014) Structural plasticity within the barrel cortex during initial phases of whisker-dependent learning. *J Neurosci* 34(17): 6078–6083.
- Landers MS, Sullivan RM (1999) Vibrissae-Evoked Behavior and Conditioning before Functional Ontogeny of the Somatosensory Vibrissae Cortex. *J Neurosci* 19(12): 5131–5137.
- Leaton RN, Borszcz GS (1985) Potentiated startle: Its relation to freezing and shock intensity in rats. *J Exp Psychol Anim Behav Process* 11: 421–428.
- Liguz-Lecznar M, Lehner M, Kaliszewska A, Zakrzewska R, Sobolewska A, Kossut M (2014) Altered glutamate/GABA equilibrium in aged mice cortex influences cortical plasticity. *Brain Struct Funct* 220(3): 1681–1693.
- Martin GK, Fitzgerald RD (1980) Heart rate and somatomotor activity in rats during signalled escape and yoked classical conditioning. *Physiol Behav* 25: 519–526.
- Mauk MD, Thompson RF (1987) Retention of classically conditioned eyelid responses following acute decerebration. *Brain Res* 403(1): 89–95.
- Mayrhofer JM, Skreb V, von der Behrens W, Musall S, Weber B, Haiss F (2013) Novel two-alternative forced choice paradigm for bilateral vibrotactile whisker frequency discrimination in head-fixed mice and rats. *J Neurophysiol* 109(1): 273–284.
- McCormick DA, Clark GA, Lavond DG, Thompson RF (1982) Initial localization of the memory trace for a basic form of learning. *Proc Natl Acad Sci U S A* 79: 2731–2735.
- Misslin R (2003) The defense system of fear: behavior and neurocircuitry. *Neurophysiol Clin* 33(2): 55–66.
- Morris RW, Bouton ME (2006) Effect of unconditioned stimulus magnitude on the emergence of conditioned responding. *J Exp Psychol Anim Behav Process* 32(4): 371–385.
- Musial P, Kublik E, Panecki SJ, Wróbel A (1998) Transient changes of electrical activity in the rat barrel cortex during conditioning. *Brain Res* 786(1–2): 1–10.
- O'Connor DH, Peron, Huber D, Svoboda K (2010) Neural Activity in Barrel Cortex Underlying Vibrissa-Based Object Localization in Mice. *Neuron* 67: 1048–1061.
- Papini MR, Bitterman ME (1990) The role of contingency in classical conditioning. *Psychol Rev* 97(3): 396–403.
- Petro N, Gruss LF, Yin S, Huang H, Ding M, Keil A (2015) Relating BOLD and ssVEPs during visual aversive conditioning using concurrent EEG-fMRI recordings. *J Vis* 15(12): 457.
- Posluszny A, Liguz-Lecznar M, Turzynska D, Zakrzewska R, Bielecki M, Kossut M (2015) Learning-dependent plasticity of the barrel cortex is impaired by restricting GABA-ergic transmission. *PLoS One* 10(12): e0144415. doi: 10.1371/journal.pone.0144415.
- Radwanska A, Debowska W, Liguz-Lecznar M, Brzezicka A, Kossut M, Cybulska-Kłosowicz A (2010) Involvement of retrosplenial cortex in classical conditioning. *Behav Brain Res* 214(2): 231–239.
- Ramos JM (2014) Perirhinal cortex lesions attenuate stimulus generalization in a tactual discrimination task in rats. *Acta Neurobiol Exp (Wars)* 74(1): 15–25.
- Rescorla RA (1967) Pavlovian conditioning and its proper control procedures. *Psychol Rev* 74(1): 71–80.
- Rescorla RA (1988a) Behavioral studies of Pavlovian conditioning. *Annu Rev Neurosci* 1: 329–352.
- Rescorla RA (1988b) Pavlovian conditioning. *Am Psychol* 43: 151–160.
- Rosen JB, Schulkin J (1998) From normal fear to pathological anxiety. *Psychol Rev* 105(2): 325–350.
- Rosselet C, Fieschi M, Hugues S, Bureau I (2011) Associative learning changes the organization of functional excitatory circuits targeting the supragranular layers of mouse barrel cortex. *Front Neural Circuits* 4: 126.
- Schneiderman N, Fuentes I, Gormezano I (1962). Acquisition and extinction of the classically conditioned eyelid response in the albino rabbit. *Science* 136: 650–652.
- Sigmundi RA, Bouton ME, Bolles RC (1980) Conditioned freezing in the rat as a function of shock intensity and CS modality. *Bull Psychonom Soc* 15(4): 254–256.
- Siucinska E (2006) GAD67-positive puncta: contributors to learning-dependent plasticity in the barrel cortex of adult mice. *Brain Res* 1106(1): 52–62.
- Siucinska E, Kossut M (1996) Short-lasting classical conditioning induces reversible changes of representational maps of vibrissae in mouse SI cortex—a 2DG study. *Cereb Cortex* 6(3): 506–513.
- Siucinska E, Kossut M (2006) Short-term sensory learning does not alter parvalbumin neurons in the barrel cortex of adult mice: a double-labeling study. *Neuroscience* 138(2): 715–724.
- Siucinska E, Kossut M, Stewart MG (1999) GABA immunoreactivity in mouse barrel field after aversive and appetitive classical conditioning training involving facial vibrissae. *Brain Res* 843(1–2): 62–70.
- Sullivan RM, Landers MS, Flemming J, Vaught C, Young TA, Polan HJ (2003) Characterizing the functional significance of the neonatal rat vibrissae prior to the onset of whisking. *Somatosens Motor Res* 20(2): 157–162.
- Thompson RF (2005) In Search of Memory Traces. *Annual Review of Psychology* 56: 1–23.
- Tokarski K, Urban-Ciecko J, Kossut M, Hess G (2007) Sensory learning-induced enhancement of inhibitory synaptic transmission in the barrel cortex of the mouse. *Eur J Neurosci* 26(1): 134–141.
- Trigo JA, Gruart A, Delgado-García JM (1999) Discharge profiles of abducens, accessory abducens, and orbicularis oculi motoneurons during reflex and conditioned blinks in alert cats. *J Neurophysiol* 81: 1666–1684.
- Troncoso J, Múnera A, Delgado-García JM (2004) Classical conditioning of eyelid and mystacial vibrissae responses in conscious mice. *Learn Mem* 11: 724–726.
- Urban-Ciecko J, Kossut M, Mozrzymas JW (2010) Sensory learning differentially affects GABAergic tonic currents in excitatory neurons and fast spiking interneurons in layer 4 of mouse barrel cortex. *J Neurophysiol* 104(2): 746–754.
- Wallace H, Fox K (1999) Local cortical interactions determine the form of cortical plasticity. *J Neurobiol* 41: 58–63.

- Weinberger NM (2007a) Auditory associative memory and representational plasticity in the primary auditory cortex. *Hear Res* 229(1–2): 54–68.
- Weinberger NM (2007b) Associative representational plasticity in the auditory cortex: a synthesis of two disciplines. *Learn Mem* 14(1–2): 1–16.
- Weiss C, Disterhoft JF (2011) Exploring prefrontal cortical memory mechanisms with eyeblink conditioning. *Behav Neurosci* 125(3): 318–326.
- Witnauer JE, Miller RR (2013) Conditioned suppression is an inverted-U function of footshock intensity. *Learn Behav* 41(1): 94–106.
- Woolsey TA, Van der Loos H (1970) The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex: The description of a cortical field composed of discrete cytoarchitectonic units. *Brain Research* 17: 205–238.
- Wróbel A, Achimowicz J, Musiał P, Kublik E (1995) Rapid phase shift of evoked potentials in barrel cortex accompanies conditioning. *Acta Neurobiol Exp (Wars)* 55: 147.
- Wu HP, Ioffe JC, Iverson MM, Boo JM, Dyck RH (2013) Novel, whisker-dependent texture discrimination task for mice. *Behav Brain Res* 237: 238–242.
- Yoshimoto M, Nagata K, Miki K (2010) Differential control of renal and lumbar sympathetic nerve activity during freezing behavior in conscious rats. *American Journal of Physiology* 299: R1114–R1120.
- Zuo Y, Safaai H, Notaro G, Mazzoni A, Panzeri S, Diamond ME (2015) Complementary Contributions of Spike Timing and Spike Rate to Perceptual Decisions in Rat S1 and S2 Cortex. *Curr Biol* 25: 357–363.