Disinhibition as the Foundation for Reinforcement in Conditioned Acquisition of Active Behaviors*

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The present paper attempts to systematize the results of our experiments concerning the mechanisms underlying the effects of reinforcement during conditioning of active behaviors. In experiments on awake rabbits by means of simultaneously records of neuron activity, slow wave biopotentials and behavior during learning, it was revealed that the pain reinforcement—UCS (unconditioned stimulus) not only evoked increased frequency of action potentials in the neurons of the neocortex and other brain structures, but also shortened inhibitory intervals and attenuated post-inhibitory rebound in response of neurons to the CS (conditioning stimuli) (flashes of light) as well as in their baseline activity. The effects of the CS, which became a signal for the aversive reinforcement, simulated this disinhibition of neuronal activity after several CS-UCS pairings. Results of special experiments showed that the reticular formation of the midbrain takes part in this disinhibitory effect. Such disinhibition results in increased regularity in the time distribution of brain neuron action potentials. Weakening of inhibitory processes facilitates transmission of excitation in the interrelated structures of the brain. Synchronous convergence of persistent regular patterns of impulses evidently plays an essential role in the processing and storing information in the CNS (central nervous system) and in execution of active behaviors.

Keywords: learning, conditioned reflex, reinforcement, inhibition, disinhibition, DSI (depolarization induced suppression of inhibition), information processing and fixation

Introduction

Investigations of behavioral phenomenology have supported the conclusion that conditioning of active and inhibitory reflexes, i.e., acquisition of new information by the CNS (central nervous system), occurs through the interaction of the fundamental neural processes of excitation and inhibition (Pavlov, 1973). The Pavlov school used the term “disinhibition” solely with reference to recovery of a reflex after it has been extinguished or in the inhibitory phase of a delayed reflex in response to an incidental stimulus. Disinhibition was not identified as a specific neural process. It was assumed that the source of this phenomenon was the spread of waves of excitation throughout the cortex, which “washed away” the inhibition, which was located at certain points in the cortex. F. P. Mayorov (1954) in his comprehensive work The History of Conditioned Reflex Theory, never used the term “disinhibition” in relation to the effect of the unconditioned stimulus. We were

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unable to locate any references to disinhibition as applied to the concept of reinforcement or of disinhibition as a process distinct from the process of excitation in works by Pavlov’s followers or those of others studying the mechanisms of conditioning. At the same time, in recent years, there has been a trend within general neurophysiology to study disinhibition as a distinct CNS process. Data showing that impairments of brain function, including such widespread disorders as schizophrenia and depression, arise, in part, as a result of a deficit in the functioning of the brain’s inhibitory systems (Lubow & Gewirtz, 1995; Luscher, Shen, & Sahir, 2011), emphasize the importance of investigating the mechanisms underlying disinhibition of behavior.

For a number of years, we have been studying the neurophysiological foundations of the interactions among the basic neural processes in conditioning using a method involving simultaneous recording of behavior, EEG (electroencephalogram), EP (evoked potentials), and the activity of individual nerve cells in the norm and after administration of various types of biologically active substances. We previously published a paper on the neurophysiological and neuromediator concomitants of the conditioned acquisition of behavioral inhibition. Experiments on awake, nonimmobilized rabbits with paws fastened to the experimental setup have shown that inhibition of the orienting reflex and of active behaviors as a result of elimination of their reinforcement, i.e., the development of internal inhibition, is accompanied by increase in amplitude of slow wave potentials in the neocortex and other brain structures and intensification of the alternation of excitation and inhibition of neuronal activity. These changes may be local, occurring principally in the cortical projection of the conditioned stimulus, or as the inhibitory state intensifies, generalized throughout the structures of the brain. These results and others in the literature have led us to the conclusion that this phenomenon is induced by the relative intensification of hyperpolarization inhibitory processes, resulting from an increase in reactivity of inhibitory systems, both local and brain-wide, to the conditioned stimulus, which has acquired inhibitory significance in the conditioning process. The fluctuations in excitability and reactivity in the population of neural elements occurring when the hyperpolarization inhibitory effect intensifies, which are dissimilar in different brain structures, play an active role in the main function of internal inhibition—limitation of excitation transmitted to effectors. The inhibitory mediator—GABA (gamma-aminobutyric acid), plays an essential role in inhibiting excitation in response to a stimulus that has lost its biological significance. These experimental results and their interpretation in the context of data from general neurophysiology suggest a hyperpolarization theory of internal inhibition (Shul’gina, 2005).

Conditioned acquisition of active behaviors is supported by other neurophysiological processes. The nature of these processes is a central issue in the neurophysiology of learning and behavior. It has been assumed that this issue can be resolved through studying modification of synapses in experiments simulating conditions of long-term use or non-use of associations among neurons, or altering synapse states by means of high frequency stimulation of nerve fibers (long-term post-tetanic potentiation or depression), etc. These studies, which are quite productive in their own way, fail to take into account the fact that the brain operates as a system. Thus, many processes that result from emergent properties of neural nets may not be observable by researchers performing model experiments. A basic hypothesis is that conditioning involves changes in the effectiveness of synapses. However, such changes require a long period of time to develop, while a conditioned reflex may form after a single pairing of the CS (conditioned stimulus) and UCS (unconditioned stimulus) and subsequently continue for a number of years (Eccles, 1964). In his book *The Physiology of Synapses*, J. Eccles (1964) proposed that during conditioning prolonged reverberatory activity arises, as a result of which a single event may activate every synaptic link in temporal-spatial structures of brain neurons thousands of times in the...
course of a few seconds. Analogous proposals have been made by other scientists (Hebb, 1949; Gerard, 1949; Konorski, 1961). Our investigations of changes in the activity of individual neurons, slow wave potentials and behavior during acquisition of active and inhibitory conditioned reflexes in alert rabbits have confirmed these ideas through actual, rather than model experiments. Moreover, we have found that there is one more important characteristic of the effects of the process of reinforcement in conditioning of active behaviors. In addition to an increase in the activation level of cortical neurons, the process of reinforcement induces disinhibition, i.e., the attenuation of hyperpolarization inhibitory effects. Detailed investigation of the neurophysiological mechanisms underlying the disinhibition process is particularly essential in light of the fact that psychological disturbances, particularly in those suffering from such pathologies as schizophrenia, are associated with dysfunctions of GABA synthesis in the cerebral cortex (Costa, Davis, Dong, Grayson, Guidotti, Tremolizzo, & Veldic, 2004; Lewis, Hashimoto, & Volk, 2005). Rats used as biological models of schizophrenia, in the presence of a deficit in latent (internal) inhibition, exhibit decrease in the number of inhibitory interneurons in the prefrontal cortex and hippocampus (Japha & Koch, 1999).

The present paper attempts to systematize the results of our experiments concerning the mechanisms underlying the effects of reinforcement during conditioning of active behaviors by considering them in the context of current results from behavioral neurophysiology, general neurophysiology, and molecular biology.

**Materials and Methods**

In our experiments, defensive conditioned reflexes were developed on alert non-immobilized rabbits attached to the experimental apparatus by four paws so that they had limited freedom of movement. In the first series of experiments, we recorded activity of neurons, slow wave potentials and behavior during the early stages of conditioned reflex acquisition. A sound or light flashes, two or four flashes at intervals of one second, served as the CS. The UCS was an ECS (electrocutaneous shock) (two or four superthreshold shocks one millisecond in duration) delivered to the skin of the hind leg at intervals of one second. The first (or the first and second) shocks coincided with the second (or the third and fourth) light flashes. Thus, in each trial, we recorded response to the CS, to the joint effects of the CS and UCS, and to the UCS alone. In the first series of experiments, 15–20 CS (sound or light flashes) were presented without reinforcement, then they were paired with the ECS, and then neuron reactions were recorded after abolition of reinforcement, i.e., during extinction of the conditioned reflex. In the next series of experiments, bioelectric indicators of brain function and behavior associated with realization of the defensive and inhibitory conditioned reflexes were recorded after preliminary conditioning of the rabbits. The conditioning process in these series experiments involved the development of conditioned defensive reflexes as in the first series experiments, and in addition, the elaboration of conditioned inhibition. The inhibitory stimulus consisted of flashes identical to the CS but presented against a background of continuous illumination (CIS (conditioned inhibition stimulus)) and was not followed by shock reinforcement. Continuous illumination began one second before presentation of the non-reinforced light flashes, signaling that the following light flashes would not precede a shock.

Glass microelectrodes filled with a 0.9% NaCl solution and with tip diameter of 5–15 μm were inserted into the brain through a 2–3 mm diameter hole in the bone using a micromanipulator which was attached to the bone. Slow wave potentials were recorded by the same microelectrode as recorded the activity of individual neurons, through attachment to another amplifier with the appropriate frequency band parameters (1–150 Hz), as well as with metal electrodes insulated with enamel implanted in the cortex. UBP1 (universal biopotential)
or UBP2 amplifiers and the universal UEF (electrophysiological) 1-03 apparatus, designed by the Central Design Bureau of the Russian Academy of Science and 8-03 USCH8 (universal eight-channel ink-writer) were used to amplify and record research data on brain function. Results were processed using raw untransformed records. In addition, we constructed peri-stimuli time histograms of reactions to the stimuli for individual neurons and for the whole group of neurons in that region of the cortex, or for groups of neurons with certain properties. Parameters reflecting neuron activity were computed (mean values of inter-impulse intervals and coefficients of variation ($C = \frac{\delta x_{\text{m}}}{x_{\text{m}}^{\prime}} \times 100\%$)). Ratios between slow wave potentials and neuron impulse values recorded at rest and during EEG activation in response to reinforcement were computed. The probability of movements in response to CS presentation, respiration frequency, heart rate, and number of movements during inter-signal periods were all computed. Reliability of results was assessed using Wilcoxon’s non-parametric test and student’s $t$-test (Bolshev & Smirnov, 1965).

In all our experiments, we recorded the activity of more than 400 neurons of the visual and sensorimotor area cortex and hippocampus in 77 rabbits. We mainly recorded the activity of neurons in the deep layers of the cortex, evidently primarily pyramidal neurons. Experimental conditions were such that we could not precisely identify the type of neurons being recorded. This is a task for future experiments.

**Results**

**The Disinhibitory Effect of Reinforcement on the Responses of Neurons to Light Flashes in the Visual Cortex**

As described in the Methods Session, activity of neurons was recorded either during an early stage of conditioning, or after preliminary acquisition of the reflexes. In both cases, we simultaneously recorded neuron activity, slow wave biopotentials, and behavior before, during, and after presentation of the stimuli.

The response of neurons to short intense stimuli in the projection cortex zones for the given stimulus (sensorimotor cortex—shock; visual cortex—light flashes) showed a temporal pattern consisting of three successive components: (1) short latency and stable activation; (2) an inhibitory delay; and (3) post-inhibition rebound of activation (see Figure 1). In the case of the light flashes (in diffuse ambient lighting), such reactions occurred in approximately half the neurons recorded. It is noteworthy that the first activation component took different forms in neurons of the visual cortex, manifesting either as high frequency flashes, or as one or two impulses, or else was completely absent. In the last case, a phase of inhibition of baseline impulse activity followed by rebound was observed.

Similar alternation of activation and inhibition phases in response to the shock delivered to a small area of the skin was recorded in the sensorimotor cortex only in infrequent cells. More often, the response consisted of a short phase of pre-excitation inhibition followed by a more or less pronounced phase of activation. Responses to continuous light or a sound in the appropriate projection areas of the cortex exhibited a tonic change in impulse frequency in either direction. Short latency stable components of the responses of neurons in the projection zones of the brain to appropriate stimuli may be tentatively described as “modality specific”, while the long latency unstable components, which generally changed over the course of conditioning, can be described as modality non-specific components of responses.

The use of light flashes in these experiments for both reinforced and non-reinforced CS proved to be a very productive device for analyzing the interaction of excitatory and inhibitory processes in the cortex of the brain. In the first and all subsequent experiments, during conditioned acquisition of the active behavior, a
fundamental property of the reinforcement procedure could be identified: Its disinhibitory effect on the activity of cortical neurons. Pairing aversive reinforcement with CS evoked either an increase in impulse frequency, or weakening of the inhibition delay and attenuation of postinhibition rebound, or both (see Figures 1 and 2 and Table 1).

**Table 1**

<table>
<thead>
<tr>
<th>Response to unreinforced light flash (the first number)</th>
<th>More frequent firing of impulses</th>
<th>Less frequent firing of impulses</th>
<th>Reaction in phases</th>
<th>Reactive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 (1 +) (1 +)</td>
<td>7 (4 +) (3 +)</td>
<td>39 (24 +) (24 +)</td>
<td>34 (16 +) (12 +)</td>
<td>87</td>
<td></td>
</tr>
</tbody>
</table>

**Notes.** In the first brackets, it is indicated the number of these neurons that responded to reinforcement with accelerated action potential: (+), with decrease of frequency action potential: (-), and with weakening of inhibitory pause: (=). In the second brackets, it is indicated the number of these neurons that responded to reinforced light flashes by imitation of effects of reinforcement.

**Response to the CS Mimics the Disinhibitory Effect of Reinforcement**

After several pairings of the shock and light flashes, which became a signal for the defensive reflex, the
neuronal responses to the flashing light were observed to mimic that of the aversive reinforcement (see Figures 1 and 2 and Table 1). It is noteworthy that the changes observed in neuronal activity were consequences of systemic reorganizations in brain structures. The nature of the response to the shock itself attests to this. In some cases, the shock itself virtually failed to activate the neuron being recorded, but rather had a clear disinhibitory effect on the combined responses to shock and light. This suggests that the disinhibitory effects of the shock result from a reaction on the part of the general activating systems of the brain, including the RF (reticular formation) of the midbrain.

The Disinhibitory and Inhibitory Effects of Modality Non-specific Influences on Modality Specific Reactions of Neurons in the Visual Cortex

Figure 3. The disinhibitory and inhibitory effect of “non-modality specific” influences on the “modality-specific” responses of neurons in the visual cortex: I: 1, 2, 3—perstimuli time histograms showing activity of various groups of visual cortex neurons: A—baseline, B—responses to light flashes, C—responses to combined effects of flashes and shock, and D—responses to shock. II: A—responses of visual cortex neurons to stimulation of the LGB, B—to joint effects of stimulation of the RF (reticular formation) of the midbrain and LGB, and C—on the RF. n—number of neurons on which the histograms are based. Histogram interval -10 msec.
We conducted a special experiment to compare the responses of neurons in the visual cortex to separate and joint stimulation of the LGB (lateral geniculate body) and RF of the midbrain (recording responses of 50 neurons) as well as the separate and joint effects of light flashes and shock (recording responses of 47 neurons) where the two had not been paired in a conditioning paradigm (Shul'gina, Oblacheva, & Lyapkusova, 1972). When modality specific stimulation of the visual cortex was paired with modality non-specific stimulation, a dual effect was observed. On the one hand, both stimulation of the RF and the effect of shock attenuated the inhibitory delay and postinhibition rebound in the reactions of the visual cortex neurons to stimulation of the LGB or to light flashes, respectively. On the other hand, the effect of these stimuli presented jointly on cortical neurons was not equal to the sum of their separate effects. Clear mutual partial inhibition was observed (see Figure 3). The differences in responses of cerebral cortex neurons in cats to separate and joint presentation of stimuli were noted by F. Morrell (1967). The inhibitory effect of a UCS on a behavioral reaction to a CS was the subject of special studies performed by the Pavlov school. It is possible that the phenomenon we observed is related to this. In any case, it is an illustration of the systemic nature of the formation of reactions in the brain even, to relatively simple stimuli. The systemic interaction of modality specific and modality non-specific effects on cortical neurons is further confirmed by the fact that shortening of the inhibitory delay and attenuation of the postinhibition rebound in neuronal reactions occurs not only in the group of cells responding to shock by more frequent emission of impulses, but also in the group of neurons that virtually does not respond to shock (see Figure 3: I: 1).

**Slow Wave Potentials and Secondary Components of Evoked Potentials in the Visual Cortex Have the Same Origin**

Visual analysis of raw recordings of slow wave potentials and neurons activity followed by statistical analysis of the relationships between these processes has shown the following. In rabbits in a state of relative rest and especially in the stage of deep extinction of a CR (conditional reflex), the EEG is polyrhythmic. Frequencies of between 1 and 10 per second predominate with the higher frequencies superimposed on the lower ones. Since this pattern of activity shows all the characteristics of human alpha-waves, it is called an alpha-wave-like rhythm. Thus, the baseline impulse activity of neurons in the neocortex and hippocampus is distributed temporally either chaotically or in the form of irregular bursts of firings, separated by inhibitory delays. In a significant percentage of neurons which of these activity types occurs, it depends on phase of slow wave potential. Statistical analysis revealed the presence of a relationship between baseline slow wave potentials and impulse activity in 22 of 87 (25.2%) neurons in the sensorimotor region and 21 of the 96 (21.9%) of the neurons in the visual region of the neocortex. In the visual cortex, these same neurons exhibited modulation of impulses in accordance with phases of late components of evoked potentials in response to the light flashes. Bursts of action potentials occurred mainly during the surface positive phase or during transitions between surface positive and negative phases in both directions (Shul’gina, 1976).

General neurophysiology tells us that the secondary components of evoked potentials and slow wave potentials arise as a result of the interaction of activating and inhibitory postsynaptic potentials involving participation of afferent, lateral, and recurrent inhibition (Eccles, 1964, 1969; Morell, 1967; Steriade et al., 1990). Thus, late components of evoked potential and EEG have a single origin and reflect the interaction of activation and inhibition processes in the cerebral cortex.
Changes in Cortical Neuron Activity During EEG Activation

The aversive reinforcing stimulus, after a number of CS-UCS pairings, also the CS, which has become the signal for UCS, induces EEG activation in the cerebral cortex. During EEG activation, we observe a neurons of the neocortex and hippocampus transition from irregular bursts or chaotic activity to the following patterns: (1) increased frequency of firing (see Table 2); (2) shortening of inhibitory delays and attenuation of postinhibition rebound (Figures 1 and 4); (3) tonic inhibition; and (4) appearance of bursts of theta waves (5–7 per second) in the hippocampus and other structures of the limbic system (see Figure 5). It is noteworthy that, during EEG activation, burst firings in a theta-wave-rhythm, such as those seen in hippocampus neurons, are not observed in the neurons of the neocortex. In some of the neurons (3.1% of 96 in the visual cortex and 12.6% of 87 in the sensorimotor cortex), there was evidence that this activity was function of theta-wave-like fluctuations occurring as a result of EEG activation. However, this activity was not of the form of burst firings, but only of some increase in the frequency of tonic impulses during a particular phase of the theta-wave. It is possible that this phenomenon is a result of modulation of impulse input to the neocortex by burst firings of hippocampus neurons in the underlying levels of the brain.

Table 2

<table>
<thead>
<tr>
<th>Brain structure</th>
<th>Sensorimotor cortex (11 neurons)</th>
<th>Visual cortex (8 neurons)</th>
<th>Hippocampus (24 neurons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High amplitude slow wave biopotential</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of interimpulse intervals measured</td>
<td>1,402</td>
<td>1,614</td>
<td>6,193</td>
</tr>
<tr>
<td>Mean interval length in ms</td>
<td>187</td>
<td>113</td>
<td>72</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>127</td>
<td>120</td>
<td>139</td>
</tr>
<tr>
<td>EEG activation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of interimpulse intervals measured</td>
<td>1,831</td>
<td>1,776</td>
<td>4,995</td>
</tr>
<tr>
<td>Mean interval length in ms</td>
<td>75</td>
<td>69</td>
<td>47</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>85</td>
<td>69</td>
<td>72</td>
</tr>
</tbody>
</table>

Increase in Regularity of Neuron Action Potential Patterns During EEG Activation in Response to Reinforcement

During EEG activation in response to the stimuli, a significant change occurred in the pattern of neuron functioning. Statistical analysis of the activity of 200 cortical neurons during periods of relative rest or of deep extinction of the CR as compared to a state during EEG activation in response to reinforcement shows that the shock evokes increased regularity in the temporal pattern of neuron activity resulting from shortened inhibitory delays and weakened postinhibition rebound. Histograms of interimpulse intervals constructed for periods of EEG activation were narrower than by the high amplitude slow wave potentials as a result of elimination very large and very small interimpulse intervals. In other words, variance of these intervals decreased (see Table 2). Increase in the regularity of neuron impulses over time was usually (but not always) associated with an increase in the mean frequency of their firing (Shul’gina, Korinevsky, & Lyapkusova, 1972; Shulgina & Korinevsky, 1973, 1975).
Thus, the reinforcing stimulus—ECS attenuates the phase structure of impulses (i.e., the alternation of activation and inhibition) not only for evoked reactions in the cortical projection of the CS—here, responses to light flashes in the visual cortex—but also for more general reactions—for generalized EEG activation. Changes in the activity of neurons in the neocortex and hippocampus in the form of tonic streams of impulses...
and in the limbic system, bursts of impulses in theta-rhythm in response UCS persisted for a long period. The short period of exposure of the reinforcer (in the majority of experiments two shocks lasting one msec with interval one second) induced EEG activation and corresponding reorganizations in the temporal pattern of action potentials lasting for tens of seconds.

**Activational and Inhibitory Types of Neuron Synchronization**

Since the initial studies of bioelectric activity of the cerebral cortex were performed, researchers have noted a rather strange phenomenon. In a state of rest animals and humans exhibit slow high amplitude potential waves, while in a state of wakeful activity, the amplitude of the biopotentials decreases and low amplitude high frequency activity is more pronounced. Among the many potential explanations of this fact, the most accepted hypothesis is that high amplitude slow wave potentials reflect the synchronic functioning of surrounding neurons, and the decrease in their amplitude in response to stimulation is associated with desynchronization of their functioning (Adrian & Yamagiva, 1935). These scientists have proposed that during a period of EEG activation neurons may operate in the same pattern as before the stimulus occurs but without synchronization of surrounding neurons. This idea has not yet been criticized and the terms “EEG activation” and “EEG desynchronization” are treated as virtual synonyms. Whether this idea is correct can be resolved through simultaneous recording of slow wave potentials and neuron activity. Our statistical analyses (based on recordings from 248 pairs of neurons in the visual cortex, 60 in the sensorimotor cortex and 48 in the hippocampus) have shown that in response to unreinforced light flashes presented during a period of conditioned inhibition, the synchronization of inhibitory type (simultaneous presence or absence of impulses) neuron activity increases in the cerebral cortex compared with baseline, reflecting a phase pattern in the activity of neurons resulting from inhibitory stimuli. The activity of these neurons is synchronized by the simultaneous occurrence of inhibitory delays. In response to combined presentation of flashes and shock during EEG activation in our experiments, in all investigated areas of the cortex, the number of neurons showing synchronic activity of the activation type (i.e., temporal coincidence of action potentials) increased compared to baseline. (Shul’gina, Balashova, & Okchotnikov, 1991). Thus, in a state of relative rest or deep extinction inhibition, there is significant correspondence in the activity of cortical neurons and slow wave potentials. Here, as has been hypothesized, synchronicity with surrounding neurons increases with respect to the type of activity associated with inhibition. In a state of wakeful attentiveness, another form of neuron activity arises, which may be called the activational type of synchronization. Both of these, in all probability, result from initiation of general brain inhibitory or activational processes, respectively. Thus, recording of EEG and neuron activity confirms only the first part of E. Adrian and K. Yamagiva’s hypothesis. During a period of high amplitude, slow wave potentials neurons do indeed operate with synchronicity. This form of synchronicity should be called the inhibitory type, since it basically arises from synchronization of discharges from neurons close to each other as a result of the occurrence of inhibitory pauses. In an active brain state, in the presence of EEG activation, the synchronization of surrounding neurons may even increase but according to an excitative rather than inhibitory pattern.

**The Functional Significance of Attenuation of Inhibitory Hyperpolarization Process in the Cerebral Cortex**

All forms of motor activity in the rabbits: orienting reflexes, responses to the UCS and to the CS, and random movements during the interstimulus intervals usually occur during periods of EEG activation,
theta-waves and increased regularity in the temporal patterns of neuron firing. We performed an experiment using animals that had not undergone conditioning in which the flashing light was presented to the rabbits either when their EEGs showed pronounced high amplitude slow wave potentials or during EEG activation induced by a shock. In the first case (high amplitude slow wave potentials), the rabbits moved in response to the flashes in 29.7% of the cases (762 stimuli presentations), and in the second case (EEG activation) in 58.3% cases (555 stimuli presentations), i.e., twice as often (Shul’gina & Korinevsky, 1975). In other species of animals and in human beings EEG activation also accompanies acquisition and performance of various active forms of behavior. It has been found that the rhythmic search (exploratory) movements of the vibrissae of rats correspond to the theta-rhythm in the hippocampus (Komisaruk, 1970), which is also directly related to support of the spatial orientation function (O’Keefe & Nadel, 1978; Shul’gina, 1976).

The Participation the Cholinergic Neuromediator System in the Effects of Reinforcement on the Cerebral Cortex

It is known that the effects of a nonspecific activating system on the cerebral cortex are transmitted by the cholinergic neuromediator system (Phillips & York, 1968; Ilyutshenok & Gilinskiy, 1971). Probably, this system also plays an active role in transmitting the generalized effects of pain reinforcement. To confirm this, we performed an experiment on rabbits with defensive and inhibitory conditioned reflexes in which the activity level of the cholinergic neuromediator system had either decreased by subcutaneous (s/c) injection of amizil (Benactyzine) in a dose of 8 mg/kg, or increased by administration of physostigmine in a dose of 0.7 mg/kg (s/c). Both drugs were dissolved in physiological saline (Shul’gina, 1986). The drugs were administered in doses that, according to data in the literature (Ilyutshenok, 1972), induce reversible changes in higher nervous activity. It was found that when Benactyzine was administered, the EEG showed stronger slow wave potentials and neurons of the cortex exhibited an increase in irregular bursts of firing, separated by inhibitory delays. Thus, they exhibited changes in bioelectric activity identical to those showed during periods of deep extinction inhibition. Administration of physostigmine was followed by EEG activation. Cortical neurons, both in baseline and in the presence of the activating and inhibiting stimuli, showed either tonic discharges or tonic inhibition of them. Movements in response to unreinforced light flashes were disinhibited. In other words, an increase in the level of cholinergic system activity induces a state in the CNS similar to that associated with reinforcement. Thus, the cholinergic neuromediator system does play a significant role in the transmission of disinhibitory effects of aversive reinforcement on the neurons of the neocortex.

Discussion

Decrease in the Amplitude of Slow Waves in the Cerebral Cortex and Generation of Theta-rhythm in the Limbic System Reflect the General Process of Information Processing

When discussing the disinhibitory effects of reinforcement, it is worth paying special attention to the phenomenon of the hippocampal theta-rhythm. This rhythm in a state of wakeful attentiveness occurs in structures of the limbic system in animals (Green & Arduini, 1964; Whishaw & Vanderwolf, 1973; Shul’gina, 1976) as well as in humans (Breeze, 1979). A number of researchers have hypothesized that this rhythm, like all other EEG rhythms, arises in consequence of the alternation between predominance of depolarization or of hyperpolarization processes, with recurrent inhibition being a contributing factor (Eccles, 1964). However, as early as 1964, Japanese researchers (Fujita & Sato, 1964) using a special method of increasing the level of Cl in the cells being recorded showed that inhibitory postsynaptic potentials do not participate in the generation of
theta-rhythm when the brain is in an active state. The rhythm is created by rhythmic transmission of groups of action potentials into the hippocampus from the septum pellucidum (Petsche, Stump, & Gogolak, 1962) and results from a fluctuation of the level of depolarization in hippocampal cells. These results suggest that both forms of EEG activation, decrease in the amplitude of slow waves and the generation of a theta-rhythm in the interconnected structures of the limbic system, result from a common process of relative weakening of inhibitory hyperpolarization effects in response to the occurrence of a new or biologically significant change in the internal or external environment. The tonic increase in the frequency of occurrence of action potentials and of burst firings in the theta-rhythm has a common property—an increase in the regularity of the temporal pattern of impulses. Evidently, it is this form of activity—long-lasting patterned streams of impulses, tonic and bursts in the interconnected structures of the brain that play a decisive role in the processes of processing and storage of new information and subsequently in its retrieval and in the performance of active behaviors.

**Neurophysiological Processes Underlying Behavioral Disinhibition**

The simultaneous occurrence of slow wave potentials and activity in certain neurons during conditioning has clearly shown that, aside from a general increase in level of activation, pairing of CS with the reinforcing stimulus induces another process—disinhibition in the functioning of neurons, manifesting itself in shortening of inhibitory delays and weakening of postinhibition rebound. Under such conditions, according to results from general neurophysiology, disinhibition at the level of behavioral reactions may be associated with various processes at the level of the systemic organization of neurons. Disinhibition of neuron activity level may be the result of a simple preponderance of excitatory influences over inhibitory ones on the neuron, or it may be a process of “inhibition of inhibition”, i.e., the result of inhibition of inhibitory interneurons by other cells of the same type (Purpura, McMurtry, & Maekawa, 1966; Tóth, Freund, & Miles, 1997). Thus, according to Tóth et al. (1997), activation of septal afferent cells initiates inhibitory postsynaptic potentials in the inhibitory cells of the hippocampus, but not in pyramid cells. These researchers hypothesized that GABA-ergic septo-hippocampal afferent cells inhibit hippocampal inhibitory cells and by this means disinhibit pyramidal cells. “Inhibition of inhibition” occurs in the brain structures that give rise to motor program, i.e., the cerebellum and basal nuclei (Eccles, 1964, 1969; Maurice, Deniau, Glowinski, & Thierry, 1998).

However, the process of disinhibition in the CNS may also be more complicated, on the one hand, and more economical on the other hand. Study of the effects of narcotics at the molecular level has revealed a process that, most likely, plays a fundamental role in disinhibition. This process has been most fully studied for endocannabinoids. It has been shown that an increase in the level of calcium in neurons as a result of their activation leads to synthesis of endocannabinoids on their membrane surface. Endocannabinoids in turn activate cannabinoid receptors the majority of which are concentrated at the terminals of inhibitory interneurons, which consequently stops emitting the inhibitory mediator GABA, i.e., lead to presynaptic inhibition of the inhibitory interneurons and thus to disinhibition of the postsynaptic neurons. This phenomenon has been termed DSI (depolarization-induced suppression of inhibition) (Scabo, Dorner, Pfereundtner, Norenberg, Starke, 1998; Kreitzer & Regehr, 2001; Wilson & Nicoll, 2001; Chevaleyre & Castillo, 2003; Diana, Levenes, Mackie, & Marty, 2002; Edwards, Kim, & Alger, 2006). It has been found that mice with a damaged receptor gene for CB1 cannabinoids habituate (i.e., develop extinction inhibition) to a new stimulus more rapidly than do normal mice (Degroot, Salhoff, Davis, & Nomikos, 2005). These researchers hypothesized that this effect is a result of enhancement of cholinergic transmission. However, a more likely cause is attenuation of the capacity of the
nervous system in such mice to produce disinhibition. It has been found that opiates inhibit GABA-ergic input to dopamine neurons of the midbrain, thus, leading to their disinhibition (Johnson & North, 1992). It has also been established that activation of muscarine receptors by an acetylcholine agonist (Carbachol) decreases the amplitude of inhibitory postsynaptic potentials (Scabo, Dorner, Pflerundtner, Norenberg, & Starke, 1998) and that introduction of acetylcholine into cells of the lateral amygdala, the nucleus accumbens and the striate body in vitro, by activating M1 receptors, inhibits release of GABA from the terminals of inhibitory interneurons (Sugita, Ushimura, Jiang, & North, 1991). Activation of glutamate receptors (kainite receptors in the hippocampus) in a similar way may mediate presynaptic inhibition of GABA release in the terminals of the corresponding inhibitory neurons (Clarke et al., 1997). In our experiments involving the administration of physostigmine, very likely, similar processes led to shortening of inhibitory intervals in impulse activity of cortical neurons and disinhibition of movements in response to inhibitory light flashes. The presence of a process in the CNS that diminishes emission of GABA from terminals of inhibitory interneurons by means of endocannabinoids and other neurotransmitters and neuromodulators of postsynaptic neurons suggests that there are a number of different causes of disinhibition on a molecular level. It must also be remembered that the effects of the postsynaptic on the presynaptic neuron may not be in the same direction in different structures of the brain. Thus, for example, it has been shown that for the terminals of striatonigral neurons, activation of cannabinoid receptors is accompanied by a reduction of reverse uptake of GABA, which tends to prolong, rather than shorten the time of its effect (Romero, de Miguel, Ramos, & Fernandez-Ruiz, 1998). It must also be remembered that the effects of endocannabinoids and opiates occur not only in GABA-ergic terminals, but also in certain brain structures with terminals of glutamatergic neurons, for example the cerebellum (Kreitzer & Regher, 2001), the nucleus accumbens (Robbe, Alonso, Duchamp, Bockaert, & Manzoni, 2001) and the hippocampus (Hajos, Ledent, & Freund, 2001). When this is the case DSE (depolarization-induced suppression of excitation) may occur.

It has been demonstrated that the depressive effect of emission of a mediator does not necessarily involve depolarization of the presynapse (the stimulation that is typically applied to cell structures to induce DSE or DSI). Generation of an action potential in the postsynaptic neuron is sufficient (Ohno-Shosaku, Maejima, & Kano, 2001). Thus, presynaptic processes participate in disinhibition. However, disinhibition does not occur as a result of axo-axonal interaction, but rather as a result of the effects of the postsynaptic on the presynaptic.

Receptors for acetylcholine, opiates, and cannabinoids have been found in all brain structures. For many years, various types of research studies have shown that EEG activation, which accompanies changes in the impulse activity of neurons, is recorded in virtually all brain structures in both animals and humans. Thus, nature provides a universal and very efficient solution to the very complex problem of eliminating inhibition when rapid spread of excitation in the CNS is essential for the organization and retention of new functional neuron systems.

That there exists in the CNS a process for weakening GABA emission from terminals of inhibitory interneurons induced by endocannabinoids and other neuromediators and neuromodulators of postsynaptic neurons suggests that disinhibition is a specific process, different in its neurophysiological concomitants from the processes of excitation and inhibition. During periods of EEG activation at the first moment of exposure to a biologically significant stimulus, cortical neurons undergo a shift from irregular bursts or chaotic discharges to increased regularity. This response provides essential information about changes in the environment and how brain functioning is organized. Subsequently, according to Shannon’s theory of information processing
(Shannon, 1963), long-lasting tonic and bursts of impulses no longer carry the new information, but, probably, have two functions: (1) maintenance of the active brain state essential for performance of ongoing actions; and (2) triggering of histochemical restructurings, in particular, those necessary for changes in the effectiveness of synapses, as hypothesized by John Eccles and others (Eccles, 1964).

We, thus, may hypothesize that disinhibition plays a decisive role in the effects of reinforcement in acquisition of active behaviors. Disinhibition, i.e., the weakening of inhibition, participates directly in processing and retrieval of information pertaining to changes in the environment that demand active behavior. Weakening of inhibitory processes in the cortex facilitate transmission of excitation in the interrelated structures of the brain. Synchronous convergence of ordered impulses enables the acquisition of information in learning. However, it should be emphasized that the nature of neurophysiological organization and the inhibition and disinhibition of behavior are heterogeneous and require further detailed study using the methods of neurophysiology and molecular biology.

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