PRECISE INSTRUMENTAL CONDITIONED REFLEXES AND THE ROLE OF DIFFERENT SENSORY MODALITIES IN THEIR EXECUTION IN DOGS

N. I. STUL

Institute of Higher Nervous Activity and Neurophysiology
Academy of Sciences of the USSR, Moscow, USSR

Abstract. Elaboration of an instrumental reaction involving conditioned rearrangement of innate motor coordination takes a long time to attain in dogs. Particular difficulties are encountered in the execution of conditioned movement when painful stimulation evokes an inborn response opposite to the conditioned one. Exclusion of visual control of performance selectively impairs the phase of searching for the "safety zone" by the animal, whereas the ability to maintain the limb in a constant position for a long time and to carry out its correct readjustments was not affected. Exclusion of afferents from the joint led to profound and irreversible motor disturbances: along with considerable impairment of the ability to maintain the limb at a given height. Also affected were searching for the "safety zone" and the ability to carry out prompt and correct adjustments upon exposure to painful stimuli. As a result, motor reactions were accompanied by a large number of shocks when the "safety zone" was wide and could not be carried out at all when it was narrow. Forcing the animals into the unusual posture strongly disrupted the elaborated reactions: the animals were no longer able to find the "safety zone" either during the isolated action of the signal or during painful stimulation. The observed disturbances tended to progress and gradually led to the derangement of the motor habit, up to complete dropping out of the conditioned reflex component.

INTRODUCTION

The behavior of man and animals consists of a number of motor habits of varying complexity, making use not only of innate, phyloge-
netically established synergies but also of those formed in the process of individual development.

The opportunity to study the basic principles governing the formation of new motor coordinations arose after Pavlov had developed the methodology of conditioned reflexes (CRs). As Orbeli (17) put it, this made it possible not only to "evolve new coordinated relations and new forms of movement but also to trace out the entire process of the reworking of coordinations through elaboration of new conditioned connections".

Experimentally, the possibility of elaborating new motor reflexes through conditioning was first demonstrated by Zeleny (26). More recently, the formation of new coordinations with the stabilization of motor CRs was investigated in detail in the laboratories of Skipin, Konorski, Asratyan and other of Pavlov's successors. The results of these studies showed the complicated nature of the relation between acquired and inborn motor responses in the formation of a complex, coordinated motor reflex. It proved impossible for the animal to execute the conditioned motor coordination or regain the old coordinations following removal of the cortical part of the motor analyzer (9), transection of the pyramids (10) or of the corpus callosum (1), or deafferentation of an extremity (16). However, these studies were mainly concerned with the coordination of non-antagonistic motor responses which could be executed by the animal concurrently. Thus, in the experiments conducted according to Skipin's method (21), lifting of the forepaw was a component of a widespread response to the electrical hindpaw stimulation, and the coordinated reaction of paw-lifting necessary to hold the food box during eating, could be executed concurrently with the unconditioned reaction (the act of eating), as shown by Ioffe (10) and others.

The present study was designed to investigate further the conditioned reorganization of inborn coordinations, that is the formation of such a complex CR which includes a movement opposite to the inborn response to the pain stimulus used.

Of special interest was to elucidate the significance of different sensory modalities for the execution of such a complex reaction, since many authors have stressed the important role of afferent input in executing complex motor acts (5, 6, 14, 15 and others), as well as the importance of proprioception (11, 16, 24, 25 and others). There have been almost no special studies into the role of different sensory modalities despite the fact that the functional organization of a conditioned movement is fairly complex and involves, along with the motor analyzer, also the visual, vestibular and cutaneous analyzers which play different roles depending on the particular form of movement. In view of this it seemed
important to compare the motor disturbances and the possibility of compensation following exclusion of kinesthetic or visual afferents and change in the animal's posture.

MATERIAL AND METHODS

In dogs, precise avoidance reflexes were elaborated. A buzzer or a tone served as conditioned stimuli (CS). The unconditioned stimulus (US) was a painful shock to the limb, evoking its unconditioned flexion. Stimulation of the skin on the back, was also used, giving a congenital response of extension of the limbs. The CS-US interval was 5 sec, and the combined presentation lasted for 10 sec. Aversive stimulation ceased when the left forepaw was lifted to a certain height — into the "safety zone". By lifting the forepaw to this height during 5 sec of the CS and holding it at the required height during the 10 sec of combined presentation of CS and US, the animal could avoid the US. The width of the safety zone was 8 cm at the beginning of conditioning and was narrowed to 4 cm later. If the dog lifted its forepaw above this zone in response to the CS, it had to lower it to eliminate the US. Thus the CR of getting the forepaw to the safety zone at the proper time could be accomplished either through flexion or extension of the forepaw depending on the initial position of the limb at the moment of initiation of aversive stimulation. In some cases the animal had, in response to the US, to perform a movement opposite in direction to the reaction inherent for the given localization of the painful stimulation.

Exclusion of visual control was achieved by conducting the tests in darkness, to which the animals were first habituated.

Sensation from the elbow joint was eliminated by local injection of 6–8 ml of 2% novocain.

An unusual posture for the animal was achieved by fixing and suspending the animal in a special stand. Rubber tubes which tightly fitted the extremities in the hip- and shoulder joints, were fixed to steel tubes attached by means of screws to the cover of the stand in which the dog was placed so that the limbs did not touch the surface of the stand. The animal was so rigidly fixed to this device that oscillations of its body in frontal and sagittal planes were completely precluded while local movement of any limb was still possible. The level of the safety zone varied depending on the level of suspension. Control tests were run in which the skin of the soles was anesthesized by injecting 2% novocain to into the toe pads. The completeness of this anesthesia was checked by determining the threshold for the withdrawal of the paw upon elec-
trical stimulation of the soles, and also by the disappearance of the placing reaction.

In order to evaluate the degree of accuracy of the motor reaction, the following parameters were used: the number of appropriately timed reactions, i.e., those in which the limb was brought within the safety zone prior to onset of the US; the number of adequate reactions, i.e., those in which the US was avoided; the duration of search for the safety zone, i.e., the time from onset of CS until paw was within the zone; the number of shocks received during the test; the mean time of corrections, calculated as the ratio of the total time per test during which the animal received the US to the number of such stimuli. By using the indices of searching time and the number of appropriately timed reactions it was possible to assess the degree of improvement of the searching phase of the elaborated movement. The number of adequate reactions and the number of times the US was received characterized the phase of maintenance of constant limb position. The mean time of corrections specified the character of the motor reaction to the US. Individual indices were averaged. The significance of differences was determined by Student's and Chi-square tests. Limb movements were recorded with an ink recorded.

RESULTS

Establishment of complex coordinated CRs

Two phases in the formation of a precise motor reaction were analyzed: (i) the establishment of this reaction during the separate action of a signal, in the absence of interference between the inborn and conditioned responses, and (ii) the conditioned transformation of the inborn reaction to the US.

Conditioned lifts of the left forepaw during the isolated action of the buzzer were observed in all dogs upon the 12–15th presentation of the CS and US. Initially, these movements were phasic and of such an amplitude that the safety zone was reached very rarely. By the 50–60th presentation of the CS, a tonic component appeared in movements in response to the signal, and gradually became dominant. However, the amplitude of the CR was in most cases inappropriate, so that the paw was either below or above the designated zone when the US began. The response to the CS attained the required degree of accuracy after 500 presentations, when in 70–80% of the trials the initial lift of the paw was sufficiently accurate to avoided the US. It was still very difficult for the animal to hold its paw within narrow limits for a long period of time. They avoided the US in only 50% of the cases (Fig. 1B). When the limb was moved out of the safety zone during the combined action
of CS and US and the dogs received the US, they always exhibited only an unconditioned flexing of the paw irrespective of its position relative to the safety zone, so that the paw was in nearly all cases much higher than appropriate (Fig. 1A).

At this stage of establishment of the motor habit, elaboration of a second reflex to another CS — tone was begun associated with electrical stimulation of the skin on the back. The first application of the tone caused lifting of the paw to the safety zone in all animals. But, as soon as the animal moved its paw outside the safety zone and so received a shock to the back, it at once made the innate reaction characteristic for this localization of pain stimulation, that is extension of the paw (Fig. 1 C). Later, this response occurred during the isolated presentation of the tone (Fig. 1D). The CR to the tone was elaborated by means of passive paw lifts and, by the 400—500th combination the same level of performance as the response to the buzzer was achieved: the animals got their paws to the safety zone in time in 70% of cases, and half of the responses were not accompanied by shocks (Fig. 1F). But, when the dogs received the US on the back, only an unconditioned extension of the limb was observed, with the paw lowered rapidly at the beginning of conditioning and ever slower later (Fig. 1E). Thus, at this phase of learning,
all animals exhibited an adequate searching for the safety zone during the isolated CS. Whatever its position, the limb moved to this zone, but only the innate reaction occurred in response to painful stimulation (flexion of the limb with US to the paw and extension upon stimulation of the back).

The conditioned reorganization of the innate reaction to painful stimulation appeared gradually. At first, the amplitude of the reaction became less dependent on intensity of the US while later the direction of the reaction was also changed. Initially, the conditioned movement took place in the presence of the innate reaction (Fig. 2A), stimulation of the back first eliciting prompt extension of the paw and only afterwards was the paw brought to the required level. Such a degree of accomplishment of motor reactions when the painful stimulus per se elicited not the innate motor response to it but rather a CR to the safety zone. In either case, US to the paw or to the back, was attained after 2,500–3,000 trials (Fig. 2BC). A characteristic feature of a well established motor habit was the performance of preliminary adjustments, i.e., correction of the limb position when approaching the boundary of the safety zone and the performance of a motor reaction in a form characteristic for a given animal, particularly when searching for the safety zone (Fig. 3).
Precise reactions of avoidance following exclusion of visual control

Despite the preliminary habituation of animals to new experimental conditions (the dogs stayed in a dark chamber for 30 min daily for 4 days prior to the experiment), considerable disturbance of the motor...
Fig. 5. Time of search for safety zone in cases of: exclusion of visual control (I), joint deafferentation (II) and posture de- ranged (III). Abscissa, numbers of experimental sessions N, control; I–III, dogs fastened to stand, but standing on floor. Arrow in III marks beginning of sessions with suspension. The dotted line denotes time of CS alone. Each point is a mean of 15 trials, vertical lines show standard deviation. A, Muk; B, Jack; C, Fomka; D, mean values for all three dogs.
reaction occurred in the first test with exclusion of visual control. Most affected was the phase of searching for the safety zone, while the animals were still able to maintain their limbs in a constant position and to perform correct adjustments in response to shock. In response to the CS, the dogs performed the established reaction, in the manner charac-

![Graphs showing reaction times and number of shocks.](image)

Fig. 6. CRs after exclusion of visual control (I), joint anesthesia (II) or postural deranged (III). A, number of shocks per experimental session; B, time of holding paw within safety zone; C, mean time of correction movement. Abscissa, numbers of experimental sessions (N and dotted line, control conditions; I-III, animals fastened to stand). Arrow marks the beginning of sessions with suspension. Filled circles, mean values for three dogs; unfilled figures, values for individual animals: circles, Jack; triangles, Muk; quadrangles, Fomka.
teristic for each of them, but did not stop their limbs in time, continuing searching movements and so receiving shocks (Fig. 4A). However, 70%/2 of the shocks were received by the dogs within the first 2 sec of US presentation, which indicated an impairment of motor reactions in the absence of vision in the searching phase rather than in the phase of maintenance of the limb in a constant position. Figures 5I and 6I show changes in the main characteristics of motor reactions with increasing number of tests in darkness. On the 2nd day, performance of the movements improved in all respects, and by the fourth session movements were nearly normal. Quantitative differences in the motor reactions in the light and in darkness were not statistically significant (Table I).

**Table I**

Mean values of the principal parameters of the conditioned reactions

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Adequate reactions (%)</th>
<th>Time of search for the safety zone (sec)</th>
<th>Mean time of correction movement (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Test</td>
<td>Control</td>
</tr>
<tr>
<td>Exclusion of visual information</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non significant</td>
<td>72 ± 3.4</td>
<td>44.5 ± 7.9</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td>Joint deafferentiation</td>
<td>67.2 ± 4.24</td>
<td>17 ± 1.23</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>Injection of physiological saline into joint</td>
<td>53 ± 6.4</td>
<td>42.8 ± 9.1</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>Postural derangement</td>
<td>65 ± 6.6</td>
<td>5 ± 2.6</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>Anesthesia of soles</td>
<td>44</td>
<td>44</td>
<td>4.9</td>
</tr>
</tbody>
</table>

**Effect of joint deafferentation on the execution of precise CRs**

Exclusion of knee joint afferents led to profound and irreversible motor disturbances. Particularly affected was the ability to maintain the limb at a given height, although the ability to find the safety zone in time was also impaired, as was the ability to perform fast and correct adjustments upon exposure to painful stimulation (Fig. 4B, 5II, 6II). The ability to maintain the limb in a given position was so strongly impaired that the dogs lowered their paws below the safety level even during painful stimulation.
With joint deafferentation adjustments gradually lost their anticipatory character acquired during conditioning, and the corrections performed upon exposure to the US became very inadequate. In most cases in response to the US totally unpredictable movements were carried out which resembled searching movements but which were usually executed either above or below the safety zone.

As a result of the above-mentioned changes, motor reactions in the presence of a broad (8 cm) safety zone were accompanied by a large number of shocks and were totally inadequate when this zone was narrow (4 cm) (Table I).

Injection of 6–8 ml of physiological saline into the joint did not have any marked effect on the preformance of precise motor reactions (Table I), ruling out the possibility that the observed changes of motor reactions were due to the injection of a large quantity of fluid into the capsule and its extensions.

**Performance of precise conditioned movements following a postural change**

In order to rule out the effect of fixing the animal to the stand preliminary tests were run in which the dogs were fixed without being suspended. Figure 5III and 6III show that the motor disturbances observed in the first test under such conditions were completely eliminated in the second and third tests.

When suspended, the dogs practically could not perform the elaborated conditioned movement (Table I). In response to a CS, they lifted the limb with a latent period characteristic for each of them. This response, however, had nothing in common with the form of the motor response characteristic for a given animal. When suspended, the dogs performed chaotic movements in response to the CS and in nearly all cases failed to bring the limb within the safety zone before they received the shock (Fig. 4C). When they received the shocks they also performed irregular movements which did not cut off the US.

As seen from Fig. 5III and 6III, the observed disturbances tended to progress, leading eventually to destruction of the motor habit up to the complete dropping out of the CR.

For a more detailed analysis of the cause of such disturbances, an attempt was made to block the pressure sensibility of the foot pads since its absence might be to a some degree a cause of disturbances when performing motor reactions while suspended. That such an effect does take place is suggested by the upset of body balance described by Orma (18) and others when the sensation from the soles was weakened, as well as by a considerable impairment of postural control (delay in
regaining the posture) when afferents from the soles were excluded by anesthesia (13). Special tests, involving, anesthesia of the soles on all limbs, showed that the absence of sensation of support in the present experiment did not disrupt the performance of precise CRs (Table I).

DISCUSSION

The necessity of transforming of innate reactions to achieve a precise CR created considerable difficulty for the animal. During the phase when a conditioned movement to the CS had already been formed, the animals were nevertheless unable to use this to halt the application of the US, which always elicited only the innate response peculiar to it. It was only gradually, after 2500-3000 trials, that the unconditioned reaction to this particular painful stimulus could be completely inhibited and a conditioned movement in the required direction and amplitude performed in response to the US.

Conditioned reorganization of inborn motor reactions may be explained on the basis of Asratyan's concept (2) concerning the formation of functional connections between different elements of the motor analyzer, making possible a motor reaction adequate to the situation. Basic to the formation of such local CR connections is a persistent increase of excitability of elements in that reaction which relieve the animal from pain. A considerable difficulty in the present experiment was the fact that this motor reaction was ambiguous in that it could be accomplished either through flexion or through extension of the limb depending on its position relative to the safety zone at the moment of stimulation. The position of the limb was a factor which determined the performance (including both the direction and amplitude) of a motor reaction adequate to the situation. Information on limb position served as a kind of CR switch determining the performance of either a flexing or extending movement in response to the same stimulus.

The need to evaluate limb position continuously, makes it impossible to execute this reaction unless complete afferent information on the course of the movement is constantly available. The experiment has revealed certain details concerning involvement of various kinds of afferents in the organization of the elaborated motor habit. Tests with novocain injection into the joints have indicated that an accurate assessment of limb position in this case is mainly made from information coming to the central nervous system from articular receptors. During the maintenance of the limb in a given position, input from the joint is either the primary source and cannot be replaced by any other type of input, or else more time is required for such replacement. This sug-
gestion seems quite plausible and agrees with numerous data on the structure innervation and physiology of the articular apparatus (4, 12, 19, 22).

The preservation, to some extent, of the ability to find the safety zone in the absence of input from the joint appears to be associated with the involvement of not only articular but also of other types of receptors in the control of performance of this phase of the motor reaction. The experiment has demonstrated the role of visual control which is selectively used only when searching for the safety zone. The need for visual control of performance has been noted by many authors (3, 23). However, the rapid adjustment to darkness shows that visual information is not the only way for the correct location of the required level. In addition to visual and articular afferents, this phase is also controlled by posture-dependent input. An unusual posture strongly and irreversibly impairs the process of searching for the safety zone, which is in agreement with the results of Chernevsky (7). This suggests that in suspended animals the functioning of the extremities is changed in a manner similar to deafferentiation. This may be explained by the fact that the absence of support for the limbs in a suspended animal changes muscular reflexes and thus alters the excitability pattern formed in the process of conditioning. The possibility of reorganization of the activity of motor units in CRs has been shown for the dog by Hori et al. (8).

It may be supposed that, when consolidated, a CR is an integral whole and includes in its structure a certain mean level and pattern of distribution of excitability of the cortical, subcortical and segmental elements whose combined activity provides the execution of the required conditioned movement of a particular pattern. A sudden change in the state of this complex of neural structures or exclusion of some particular type of afferent control makes it impossible for the animal to perform the CR with the required degree of accuracy. The significance of different sources of information depends on the degree of reaction stabilization, on the given parameters of movement, and even on the individual characteristic of the animals.

REFERENCES


Received 3 July 1972

N. I. STUL, Institute of Higher Nervous Activity and Neurophysiology, Academy of Sciences of the USSR, Pyatnitskaya 48, Moscow, USSR.