EFFECT OF NICOTINE UPON THE REFLEX ACTION OF SOME CUTANEOUS SENSE ORGANS IN THE FROG.

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The basis of this investigation was to determine the effect of nicotine upon certain skin reflexes in the frog: to determine the duration of this effect; its after effect; if immunity could be established; and how the action of nicotine compares with that of alcohol. The experiments were conducted upon frogs af the species Rana Pipiens, secured from a near by lake. They weighed from 15 to 120 grams, and during the period of experimentation were kept in a dark room in cool moist moss, without food.

The sensory ending of the cutaneous nerves may be considered peripheral end organs. By carefully testing certain spots, some were found which responded with a fairly constant reflex time, to a definite stimulus. The same spot varies somewhat at different times of the day and also at different room temperatures; on cool days the response was quicker.

The spots selected for study were those which proved after careful testing to be most reliable and constant in their reaction time. These spots are illustrated in text, fig. 1, and were the same ones employed in a former experiment.[1]

The constant stimilus determined upon, was one that would not injure or fatigue the peripheral nerve endings during the period and method of experimentation. Pure neutral filter paper, three millimeters square moistened with 8 per cent pure acetic acid was found the most practical chemical stimilus. The paper was moistened in the acid, then placed carefully by means of a long forceps upon the spot to be tested. That the errors due to sight and pressure stimuli were avoided, was proved by control experiments where eyes were covered by a special devise.

By reflex time is meant the interval between the moment the paper touches the skin and the moment the frog made an attempt to remove it. It was found that if the attempt was not made within one minute, it usually never would be. As soon as an attempt was made or if not made at the end of one minute, the acid paper

1 Am. Journal Phy. Vol. 31 p. 309. 1913.

was washed off with fresh water thus preventing fatigue and injury to the peripheral nerve endings. This was proved by testing the corresponding spot on the opposite side and also by control experiments on frogs which had not been given nicotine.

Control experiments were made by injecting Ringer's solution in amounts similar to the largest ones of the drug, to determine the mechanical effect of injection and also that of dilution on the circulating fluids. The doses employed varied from ½ minims of 0.05 per cent per 10-gram frog, up to 2½ minims of 0.1 per cent 10-gram frog, which proved to be a just toxic dose for a normal frog. The nicotine was diluted with distilled water and was injected subcutaneously into the dorsal lymph sac. From a former experiment we found this method most satisfactory.

In carrying out an experiment, the reflex time of the series was found upon the frog before nicotine was given, then the drug was injected and 10 minutes were allowed to overcome the mechanical effects before testing the spots again. This testing was repeated every ten minutes for about two hours

or until the reflex response returned, allowing an interval of 10 minutes rest between each test. If the reflex response did not return at the end of two hours, the tests were repeated the following day. The acid paper was applied with one hand and the stop watch was used with the other to record the reaction time. Philips and Pembry[1] give 10 minnims (.59cc.) of 1 % part of nicotine in 20 parts saline solution as a, toxic dose and Cushing, 1cc. of .2% solution. ~~My nicotine was made up with distilled water, and perhaps these were not just toxic doses.~~ The reflex time and effects secured after injection of nicotine were compared with the reaction time and effect obtained before injection, also with the reaction time after injection of Ringer's solution. From these observations the effect of nicotine upon the frog was deduced.

It was found that each spot had its own reflex time, its own degree of irritability and that some were more resistant to nicotine than others. Spot C, (figure 1, and Table I) is normally irritable, usually responding to the acid stimulus in one second, and after doses large enough to cause a loss of reflex, was always the first to recover; often being the only spot to give a reaction

1. Physiological Action of Drugs. P. 58. 1901.

SENSORY SPOTS.

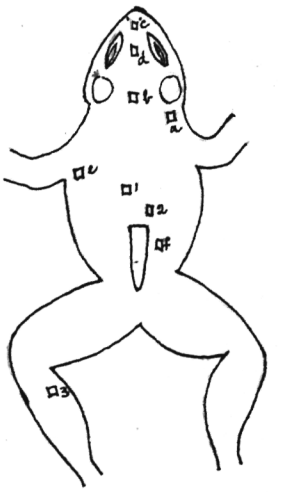

Figure I.

Diagram showing location of sensory spots on the frog. Those on the head are innervated by branches from the cranial nerves, those on the trunk and legs by spinal nerves.

response. Spots f. and c. also respond quickly to the acid stimulus and recover quickly from the seemingly paralysing effect of the nicotine. Spots i. and e. seem to be the least irritable and show the least resistance to the action of the drug. It was found that spots c. 3, and f. were the most irritable normally and showed the most resistance to the action of the drug. Table I also shows that nicotine has both a paralysing and a depressing effect. First it paralyzes the ganglia cells, (according to Langley), then as soon as the paralyzing effect passes off, there is a depression of the spot, making it slower in its response to the acid stimulus shown in Column II and I. Column III shows the duration of paralysis in the different spots, due to the doses indicated. For instance spot c. lost its reaction time for 120 minutes, while b. lost it for 360 minutes.

It was interesting to note that as weak a dose (Table I, III, A.) of nicotine as ½ minim of 0.05 per cent per 10-gram frog produced a change in the reflex time of the cutaneous sensory spots, but not any apparent effect on other reflex actions, such as turn over, com-

pensatory, and swimming, or upon the general behavior of the frog. It had a more depressing effect than a larger dose of Ringer's solution. With this dose, spot c. was the only one which did not lose its irritability or fail to respond to the acid stimulus at any time. S mall doses from ½ to 5 M per 10-gram frog were followed for about 15 minutes by slightly forced or labored breathing, and also by a slight constriction of the pupils. Weak fibrillary twitchings were also noticed immediately after injection.. These lasted only for a short time, during which the position was usually normal.

With medium doses, (Table I, III, b.) the skin reflexes were all lost and for a longer time, the breathing became more forced, the constriction of the pupils more apparent, and the fibriller twitchings more pronounced. The frog assumed a flattened position, and as soon as the deepened breathing became normal again, seemed sluggish.

With large doses, (Table I, III a) i.e. of 10 minims of 0.05 per cent per 10-gram frog and over, the higher reflexes as well as skin reflexes were lost for 1 hour or longer. It was noticed that when the spots

COMPARISON OF IRRITABILITY OF SPOTS.

	I	II	III		
Spots	Reaction Time Before Nicotine	Reaction Time After Nicotine	Length of Paralyzing Effect		
			.5 m (a)	6 m (b)	14 m (c)
1	2 seconds	4 seconds	40 minutes	100 minutes	180 minutes
2	12 "	20 "	40 "	100 "	180 "
3	2 "	4 "	20 "	80 "	120 "
a	7 "	8 "	20 "	100 "	360 "
b	8 "	38 "	40 "	150 "	360 "
c	1 "	1 "	10 "	50 "	120 "
d	5 "	5 "	20 "	50 "	120 "
e	15 "	30 "	40 "	150 "	360 "
f	4 "	10 "	20 "	75 "	180 "

Table I.

For location of spots, see text, Figure 1.
M. Minims, 1 drop. .016 cc.
Stimulus - acid paper, see text.
Nicotine of 0.05 per cent.
Column I show reaction time to an acid stimilus for a normal frog.
Column II shows the immediate depressing effect of nicotine after paralyzing effect has passed off.
e. g. Spot 1 in a normal frog responded to the acid stimulus in 2 seconds after the paralyzing effect, that lasts for 40 minutes had passed off, its reaction time to the stimulus increased to 4 seconds.
Column III shows the length of time that the paralyzing effect of a weak, medium, and strong dose lasted for the different spots.

failed to respond after a dose of nicotine, they did did so immediately. The paralyzing effect increased with increase of the dose. The frog returned to a seemingly normal state within two days, but the skin reflexes usually desplayed an increased irritability for some time. There was a period of inhibition of respiratory movements at first, the length increasing with an increase of dose. With the largest doses, this inhibition lasted for 2 hours or longer and was followed by forced irregular breathing.

The large doses also caused an immediate stiffening or tetanus of the front legs, which lasted about 15 minutes. This stiffening spread somewhat to the trunk and hind limbs, then was followed by a relaxation and loss of muscle tone, and continued until the higher reflexes again made their appearance. From these observations we conclude,

1. That nicotine causes a loss of the skin reflexes, a seemingly paralyzing effect that is followed by one of depression.

2. That nicotine in small doses caused forced breathing; in large doses an inhibition of respiratory

movements, followed by forced irregular breathing.

3. That it causes fibrillar twitchings and constriction of the pupils.

4. That large doses cause tetanic contraction of the front legs, and sometimes a slight stiffening of the whole body, followed by relaxation and loss of muscle tonus.

II TOLERANCE EXPERIMENTS.

In connection with the above observations, a series of experiments were carried on to determine if tolerance to the drug could be established. A number of frogs were given first a minimum dose, ½ minims of 0.05% per 10-gram frog, then at intervals of ~~one~~ or two days were again injected with ~~slightly increasing~~ doses of 1 minim until the last dose given proved toxic to a normal frog.

Table II

Comparison of Normal and Tolerance Frogs

Frog	Dose per 10-grams	% Nicotine	Skin Reflexes Lost	Higher Reflexes Lost	Irritability	Constriction of Pupils	Position	Behavior	Respiration
Normal	.5 m	0.05	15 min.	No Loss	None	very slight	normal	Normal	Deeper
Tolerance	.5 m	0.05	No Loss	"	very slight	" "	"	"	"
Normal	3 m	"	32 min.	"	none	slight	not "	Slight Twitchings	Forced
Tolerance	3 m	"	No Loss	"	slight	"	"	Normal	"
Normal	6 m	"	More than 2 hrs	"	none	constricted	flattened	Twitchings	" "
Tolerance	6 m	"	No Loss	"	quite	"	slightly flattened	Normal	"
Normal	10 m	"	6½ hours	60 min	sluggish	Very constricted	very flat	Twitching	Inhibition followed
Tolerance	10 m.	"	83 min	60 "	"	" "	" "	No twitching	by irregular
Normal	14 m	"	more than 12 hrs	more than 4 hrs	"	" "	slightening of front legs	No twitching	Inhibition at first. then
Tolerance	14 m	"	125 min.	95 min.	Loss of mu. Tonus	" "	" "	" "	Irregular
Normal	18 m	"	more than 12 hrs	more than 6 hrs	Not very	Very constricted	very flat	Twitchings follow injection	Apparent inhibition for some time
Tolerance	18 m	"	" " 12 hrs	" " "	Very irritable	" "	" "	" "	" "
Normal	7 m	0.1	about 12 hrs	more than 6 hrs	not very	" "	" "	" "	Inhibited followed by irregularity
Tolerance	7 m	0.1	never returned	never returned	Lost	" "	" "	" "	Never recovered
Normal	9 m	0.1	never returned	never returned	"	" "	" "	" "	" "
Tolerance	9 m	0.1	" "	" "	"	" "	" "	" "	" "

To prove that laboratory conditions and fatigue due to acid tests were not factors to be considered, control frogs were placed under the same laboratory conditions and subjected to the acid tests as frequently, and on the same spots as were those employed in the tolerance experiments.

Table II shows the typical effects of equal doses of nicotine upon a normal frog and upon one in which tolerance is being established by inflicting gradually increasing doses with it has begun. In comparing the effects of nicotine on a normal frog with that exhibited by a tolerant one, it was seen that with small doses, there was considerable difference in the effect upon the two frogs. The normal one lost all the skin reflexes for a time and exhibited fibrillar contractions. The only apparent effect upon the tolerant frog was the forced breathing and increased irritability. Its skin reflexes all responded to the acid stimulus at the end of 10 minutes. With medium doses, 10 minims of 0.05% to 7 min 6.1% per 10 grams frog. ~~large enough to cause loss of the higher reflexes~~, the most noticeable difference was that, altho both frogs passed thru the different stages produced by nicotine, they did not last so long on the tolerant frog and its skin reflexes responded much more quickly to the acid

stimulus. It was interesting to see that sometimes the skin reflexes responded even before the higher reflexes returned, this being contrary to the usual events.

Other interesting observations taken on the tolerant frog were the facts that after recovery from the effect of the drug, the animal became very irritable and the spots responded abnormally, all of them within one second. Often the slightest touch on the skin called forth a violent reflex movement. The tolerant frog also began to show a yellow discoloration of the skin on the under side of the lower part of the body and hind legs. Altho this discoloration remained permanent, it was most noticeable shortly after injection. It was also found that there was a decided loss of weight of the frogs which were given almost daily doses of nicotine, while the control frogs showed but slight loss, due perhaps to lack of food.

Some of my results corroborate those published by Langley[1], who found that in the skate, 1 per cent nicotine has an extraordinarily strong and local effect upon the bulb and cells in the sympathetic ganglia, also that the application of nicotine to the spinal bulb

causes for a time, cessation of the respiratory movements in consequence of the tetanic contraction of the muscles; the muscles then relax and feeble respiratory movements occur. He explains the fibrillary twitchings by the facts that nicotine is a stimulant to the motor nerve cells of the bulb and spinal cord. After this stimulation however, there is a paralyzing effect. I also found as did Chaney and Sollman, that nicotine caused a contraction of the pupil.

In comparing the results exhibited by nicotine with those found in a previous experiment with alcohol,(3) I found that the differences are quite marked. Both cause a loss of the skin reflexes and in sufficient quantities, loss of the higher reflexes, but nicotine is much more powerful. A dose of 0.008 cc. of 0.05% nicotine per 10-gram frog affects the animal more intensely than a dose of 0.005 cc. of 15% to 30% alcohol per 10-gram frog. Nicotine produces a slight stimulation at first, alcohol never stimulates. Alcohol did not affect the respiration or cause constriction of the pupils. But in large doses, it too caused tetanic contraction of the whole body and death. Nicotine causes

a peculiarly characteristic tetanus of the front legs followed by a relaxation and loss of muscle tonus.

This investigation was undertaken at the suggestion of Dr. Ida H. Hyde, to whom I am greatly indebted for constant supervision and help.

SUMMARY.

1. Certain sensory spots in the frog's skin differ not only in irritability and reflex action, but also in susceptibility to the influence of nicotine.

2. The skin reflexes are affected by much smaller quantities of nicotine than are the higher reflexes, turn over, compensatory, and swimming.

3. Small doses of nicotine cause a depression or loss of the reflexes, fibrillar contractions, forced respiration and a slight constriction of the pupils. One dose did not usually show an increased irritability as an after effect. These changes appear immediately after injection and last from one-half to three or four hours depending upon the dose.

Doses of ½ minim of 0.05 per cent per 10-gram frog have a greater effect than larger doses of Ringer's solution. Large doses of nicotine cause an entire loss of the skin reflexes, and muscle tone, producing a flattened position; an inhibited then irregular respiration; constriction of the pupils; loss of turnover, compensatory and equilibrium reflexes; a tetanus or stiffening of the front legs, followed by a relaxation and loss of muscle tonus.

4. Continued gradually increasing injections of nicotine cause tolerance to the drug, such that it loses some of its effect. Continuel use of nicotine causes increased irritability of the skin reflexes, making them respond abnormally. It also causes a loss of weight, and a discoloration of the skin. The effect upon respiration and constriction of pupils is the same as in a normal frog, except that the effect does not last so long.

5. Nicotine is much more powerful in its effects on cutaneous reflex reactions, ciliary muscles, respiratory activity and bulbar centers than is alcohol in even larger doses.