J. Exp. Biol. (1969), 50, 515–526
With 2 text-figures
Printed in Great Britain

THE EFFECT OF SPLITTING PART OF THE BRAIN OR REMOVAL OF THE MEDIAN INFERIOR FRONTAL LOBE ON TOUCH LEARNING IN *OCTOPUS*

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(*Received* 24 *July* 1968)

INTRODUCTION

The object of these experiments was to get further information about the parts played by the various supraoesophageal lobes in touch learning. The anatomy is described elsewhere (Young, 1964, 1969). We have compared the rate of learning a simple successive tactile discrimination task in animals with (1) no central lesion ('normals'), (2) the whole supraoesophageal brain divided in the mid-line ('half-brains'), (3) the whole inferior frontal system divided ('anterior splits'), (4) the rest of the supraoesophageal lobes divided, leaving the inferior frontal system intact ('posterior splits'), and (5) with the median inferior frontal lobe removed.

The animals were trained on one side and in most of them the opposite side was later tested for its capacity to discriminate. It has already been shown that in animals trained on one side after complete supraoesophageal section in the mid-line the untaught side does not discriminate (Wells & Young, 1966). The present experiments show that the passage for side-to-side transfer is mainly through the inferior frontal system, and particularly through the median inferior frontal lobe. The vertical lobe system possibly provides a channel for some lateral transfer. The experiments also show that after removal of the median inferior frontal lobe touch-learning is grossly impaired

METHODS

The octopuses were between 250 and 450 g., obtained from the Bay of Naples and kept separately. They were tested with crabs on the days after capture and operated when they were feeding regularly. Under urethane anaesthesia all were blinded by bilateral section of the optic nerves. In those to be 'normal' controls the cranium was then opened and the jelly removed. In the 'operated' animals bisections were performed with a fine scalpel blade. Scalpel and very fine scissors were used to remove the median inferior frontal lobe.

In the days after operation each animal was tested 8 times morning and evening with a small piece of sardine, given on a wire. Training was begun only with animals that were feeding regularly. Rough and smooth plastic spheres were used as already described (Wells & Young, 1966). There were two training sessions each day. At each there were eight trials with the positive and eight with the negative sphere, given alternately with positive first. Food was given only when the positive sphere was passed towards the mouth or when it was still being held by the suckers after 20 sec. A shock (10 V. a.c.) was given when the animal similarly took the negative sphere or held it for 20 sec. Trials were at intervals of about 5 min. All training was on the right side, using the second arm whenever possible.

Each animal was trained on the right side for a total of 160 trials (80+ and 80−). Then training was continued for a further 80 trials interspersed with unrewarded tests on the left side using the same spheres. In order to avoid the effects of food and shocks given in training the tests on the left side were given in the order

$$+(+)-(-)+(-)-(+)+(+)-(-)+(-)-(+)$$

(where the tests are in parentheses). On the next day the test sequence was reversed: $+(-)-(+)+(+)$...and so on.

At the end of the experiment the brains were fixed in formalin, silver-stained in block and serially sectioned (Stephens, 1969).

The temperature of the sea water varied from 23° to 26° C.

Normal octopuses have a greater tendency to take smooth than rough objects and this difference is reversed after some lesions (Wells & Young, 1968). In the present experiment, therefore, half the animals with each operation were trained in each direction, selected so far as possible randomly out of each batch operated.

RESULTS

Comparison of rate of learning of normal and half-brains

The normal animals showed a clear preference for smooth (Figs. 1, 2). The details of their performance are recorded elsewhere (Wells & Young, 1968). The split animals trained with smooth positive began at a lower level of smooth preference than normals (52% against 64% takes of smooth in first 16 total takes) but thereafter the two groups learned at essentially the same rates. The half-brains trained rough-positive began with a somewhat greater tendency to take rough than normals and this difference remained throughout. The main difference between normal and split-brain animals is thus that the former have a smooth preference but not the latter. The rate of improvement in discrimination is similar in both. There was, however, a somewhat greater tendency to take both objects in the half-brain animals, perhaps because they lack the vertical and subfrontal lobes of one side. There is evidence that these lobes have in general an inhibitory action. In tactile experiments animals typically begin by taking both of the objects to be discriminated. Training usually results in a diminution of the proportion of objects taken, at first indiscriminate but later limited to the negative object. Removal of the vertical and subfrontal lobes tends to lead to an increased proportion of takes.

These results are in apparent disagreement with some previously reported in which half-brain animals trained with smooth positive were considered to learn more slowly than normals (Wells & Young, 1966). However, in that experiment, as in the present one, the half-brains took fewer smooths than did the normals over the early trials, the effect of a shift in untrained preference that is associated with the splitting operation and which was only detected subsequent to the 1966 experiments (Wells & Young, 1968). When the results with animals trained S+ and R+ are considered together, normal and split-brain octopuses show a similar rate of improvement in this simply discrimination.

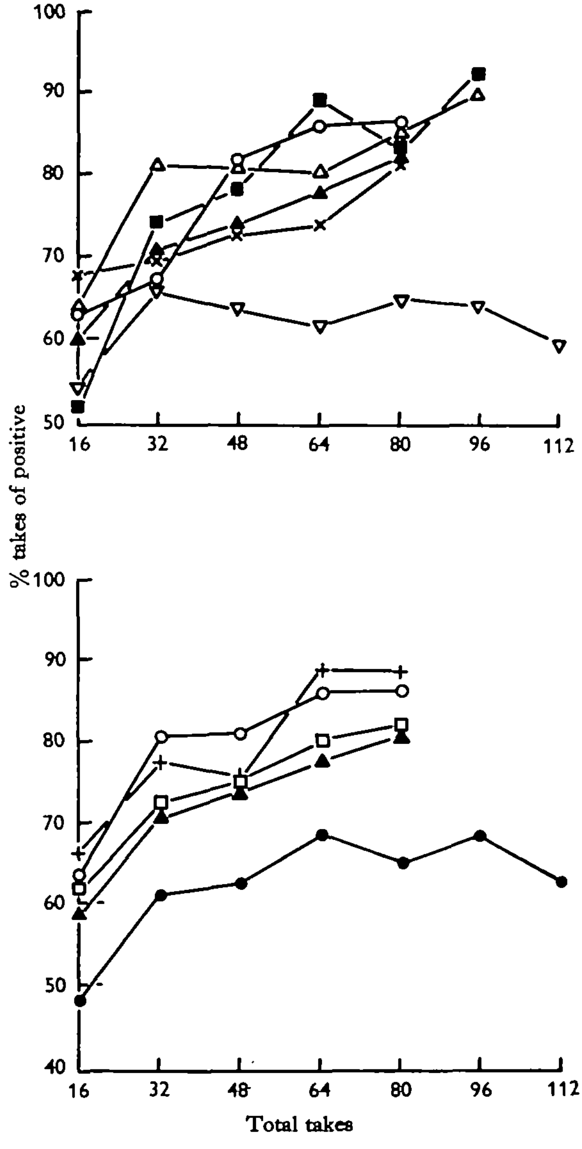

Fig. 1. Mean percentage of takes of the positive object for each 16 total takes. The various groups plotted separately, with the performance of normal animals plotted in both series to make comparison easier. ○, Normal S+ ($n = 9$), ▲, normal R+ (7); ■, half-brains S+ (9); △ half-brains R+ (9); □, anterior split S+ (6); +, anterior split R+ (5); ●, posterior split S+ (6); ▽, med. inf. fr. removed S+ (6); ×, med. inf. fr. removed R+ (7).

Inferior frontal system only divided

Eleven animals were satisfactorily trained on the right side with the inferior frontal system split (Table 1). There was no serious damage to the right subfrontals and only

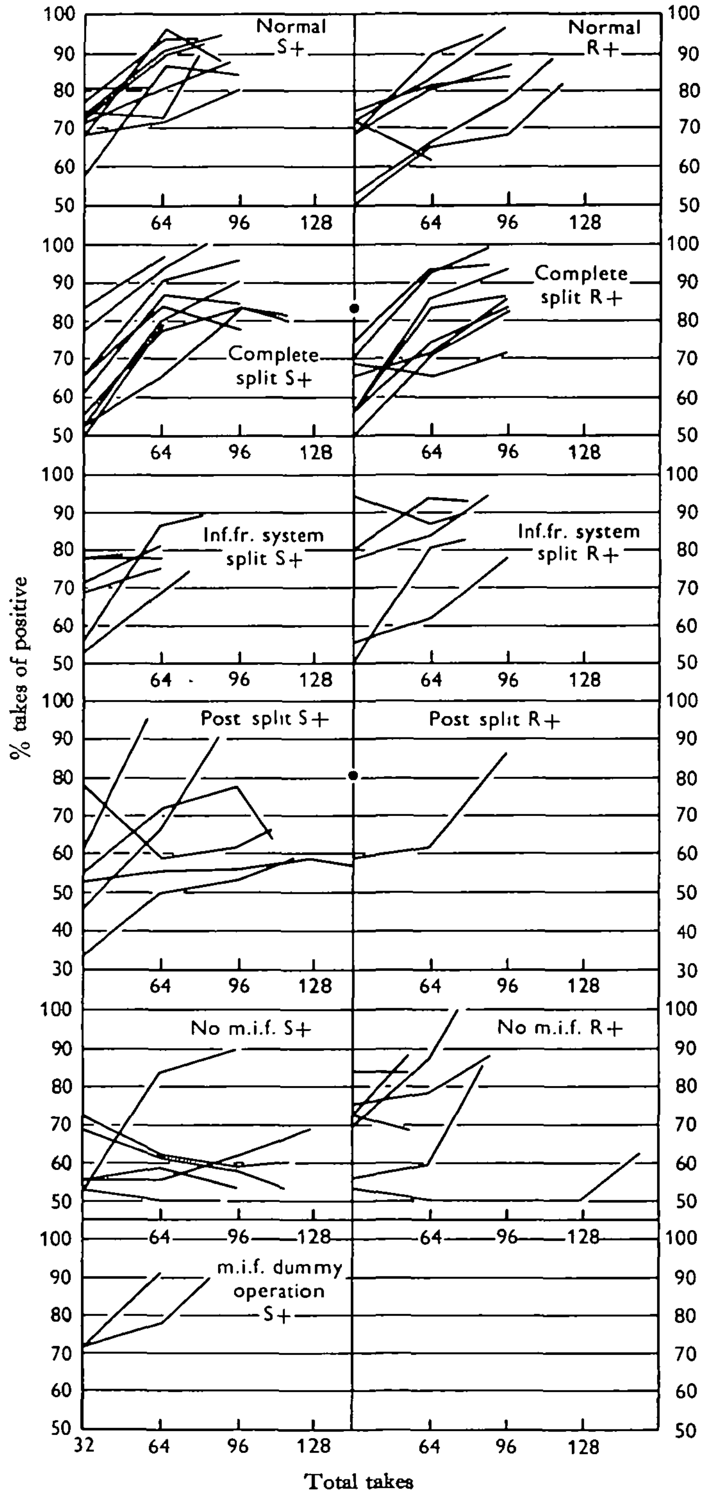

Fig. 2. Percentage takes of positive object for each 32 total takes shown for each individual animal. The filled circles indicate animals that did not take at more than 32 trials.

in one (slight) to left subfrontal. The median inferior frontal and subfrontal commissures were completely cut except for some fibres of the latter in one animal.

The right sides all learned well, with correct response means of 71·5% S+ and 80·0% R+ over 160 trials. In 80 further training trials they gave 77·8% S+ and 93·2% R+ (Table 1). These animals therefore prefer rough (Figs. 1, 2). It would be interesting to know whether these half-inferior-frontals learn at a different rate from normals, but comparisons are of course made difficult by the fact that the half-brains have a preference opposite to the normals. If we take the mean correct response for rough-positive and smooth-positive together we obtain 75% over 160 trials for the 23 normal animals already reported (Wells & Young, 1968) and 76% for the present 11 half-inferior-frontals. Of course such a comparison implies that the preferences are equally strong in the opposite directions, which is unlikely to be true. Even so it is clear that no great deficit in discrimination learning is involved by halving this part of the system. This is confirmed by comparing the scores achieved in each successive series of 16 takes by the different groups of animals (Fig. 1). The differences in preference appear throughout but the rate of gain of information is in general similar in normal animals, in those with the supraoesophageal lobes wholly split and in those with the anterior part only divided. The course of learning was reasonably consistent in the different animals with anterior splits (though two out of the eleven animals showed a clear preference from the start: one for S+, one for R+), which was not modified by subsequent training.

The animals with anterior splits, when tested on the left, showed only faint signs of correct response. Ten out of the eleven gave 50% correct or more, but only three were over 60% correct. The means were 57% for R+ and 56% for S+. The small sign of capacity for correct response on the untrained side can perhaps be taken to show that some transfer takes place through the posterior part of the brain. It must be remembered, however, that the risks of contamination are great. If a sphere is carried rapidly towards the mouth it is difficult and sometime impossible to prevent it touching arms on the other side near the mouth. In a previous series of experiments using exactly the same training techniques there were, however, no signs of transfer at all after complete splitting (Wells & Young, 1966). This previous study also included five animals with the inferior frontal system alone split and they showed even more correct response on the (untrained) left side than the present series (73%, all with smooth-positive). It seems likely, therefore, that some transfer can take place in these animals, with the inferior frontals split, presumably through the vertical lobe system.

An interesting feature of the animals with anterior splits is the very low number of takes of the 'positive' on the left compared with the very high number on the right. Clearly there is 'positive' learning by the trained side as a result of the food taken, and this has not been transferred. Every single animal shows much higher takes of the positive on the right than left (Table 1). Conversely, in all animals except two there were more takes of the negative on the left. The contrast is very striking and this is excellent evidence of learning on both directions. Very little of either can transfer through the vertical lobes or be used by the opposite side through them.

Table 1. *Results of splitting the whole inferior frontal system, leaving the posterior part of the supraoesophageal lobes intact*

(Animals first trained on right side then tested on left while training continued on right. The lesions are represented on a four-point scale, with 0 indicating no damage and 3 complete removal (or severance of a commissure); msf., median superior frontal lobe; mif., median inferior frontal lobe; subfr., subfrontal lobe; dors., dorsal; ventr., ventral; R, rough; S, smooth; '%A' means attacks at positive stimulus as percentage of presentations of positive. %C mean correct responses as percentages of all presentations.)

	Train right (160 trials)				Train right (80 trials)				Test left (80 trials)				Amount			Damage to subfr.			
																L.		R.	
	S+	R−	%C	%A	S+	R−	%C	%A	S+	R−	%C	%A	Split msf.	Split mif.	Split Subft.	dors.	ventr.	dors.	ventr.
									Smooth +										
408 = PGD	48	28	62	58	27	15	69	68	15	19	45	38	0	3	3	1	1	0	0
413 = PGH	59	19	75	75	38	24	68	95	21	14	59	53	3	3	3	0	0	0	0
422 = PGK	60	16	78	75	38	16	78	95	26	14	65	65	3	3	3	0	0	0	0
453 = PGO	36	10	66	45	30	6	80	75	18	15	54	45	0	3	1	0	0	0	0
454 = PGP	63	20	77	79	39	13	83	98	25	13	65	63	0	3	3	0	0	0	0
458 = PGR	51	19	70	64	40	9	89	100	17	13	55	43	0	3	3	1	0	1	0
n = 6	317	112	71·5	66·2	212	83	77·8	88·5	122	88	57·2	51·2							
									Rough +										
	R+	S−			R+	S−			R+	S−									
418 = PHC	71	13	86	89	40	4	95	100	20	20	50	50	0	3	3	0	0	0	0
420 = PHD	70	9	88	88	36	4	90	90	14	7	59	35	1	3	3	0	1	0	0
424 = PHE	68	7	88	85	39	3	95	98	4	4	50	10	1	3	3	0	1	0	0
482 = PGW	66	36	69	83	37	7	88	93	33	20	66	83	2	3	3	2	2	0	0
490 = PGY	52	24	68	65	38	0	98	95	19	15	55	48	0	3	3	1	0	0	0
n = 5	327	87	79·8	82·0	190	18	93·2	95·2	90	66	56·0	45·2							

Table 2. *Animals with back of supraoesophageal lobe divided, but inferior frontal system intact*

(Training and abbreviations as Table 1. Vert. = vertical lobe. Damages to the median superior frontal and vertical lobes are rated on a 5 point scale.)

	Train right (160 trials)				Train right (80 trials)				Test left (80 trials)				Lesion msf.		Intact vert.	
	S+	*R*−	% C	% A	S+	R−	% C	% A	S+	R−	% C	% C	L	R	L	R
							Smooth +									
403 = PGC	56	30	66	70	33	15	73	83	28	11	71	70	3	2	3¼	1½
410 = PGF	57	61	47	71	31	23	60	78	7	5	53	18	3	2	3¼	¼
412 = PGG	80	62	61	100	37	31	58	93	21	21	50	53	3	2	4	1
414 = PGI	73	35	74	91	25	6	74	63	25	7	73	63	2½	2½	2½	2¼
450 = PGN	45	13	70	56	40	10	88	100	29	3	83	73	3	2	2¼	2
455 = PGQ	75	35	75	94	37	23	68	93	33	14	74	83	4	1	3½	1¼
n = 6	386	236	65·5	80·3	203	108	70·2	85·0	143	61	67·3	60·0				
							Rough +									
	R+	S−			R+	S−			R+	S−						
437 = PHF	33	11	65	43	34	12	78	85	31	22	61	78	Not divided		2½	2½
481 = PGU	73	30	76	90	39	7	80	98	37	35	53	93	4	1	3½	1½
n = 2	106	41	70·5	66·5	73	19	84·0	91·5	68	57	57·0	85·5				

Posterior supraoesophageal lobes split, inferior frontal intact

There are eight of these: six S+ and two R+ (Fig. 2.). In general these learned on the right side to levels of correct response *lower* than the anterior splits (Figs. 1, 2). The difference is due to a greater tendency to take both positive and negative in the animals with posterior splits.

		+	–
Anterior split	S+, $n = 6$	317	112
	R+, $n = 5$	327	87
		644	199
		72·5 % of trials	22·6 % of trials
Posterior split	S+, $n = 6$	386	239
	R+, $n = 2$	106	40
		492	279
		76·8 % of trials	43·6 % of trials

The difference in attacks at the negative is significant at 5 % level (Wilcoxon $t = 53$, $n_1 = 8$, $n_2 = 11$). This is good evidence that removing half of the vertical lobe causes increased takes especially of the negative object. Actually removal was of more than half, since many of the cuts lay to the right of the mid-line (see Table 2). The large number of takes of both spheres continued in the second period of training throughout the period when the animals were also getting tests on the untrained side.

Tests on the left side showed more correct responses on the untrained side than with the anterior splits (67·3 % correct S+ and 57·0 % R+). There were, however, three octopuses out of the eight posterior splits tested that made less than 60 % correct response and the mean difference between anterior and posterior split-brain animals is not significant at the 0·05 % level. There are reasons for the three failures to show good response on the untrained side (Table 2). Octopus PGI learned very poorly on the right and should probably be excluded altogether for that reason. PGG was a very strong taker and correspondingly poor discriminator. PGW was such a strong taker on the left that it took nearly everything. We can therefore be confident in saying on the basis of the remaining six animals that with a split vertical-lobe system correct response can take place on an untrained side. Therefore one must suppose either that the memory record is transferred in the more anterior lobes, through one of the two inferior frontal commissures, or that the untrained side uses the record in the trained side through one or both of these (see Wells & Young, 1966).

This transfer, or capacity to read out from the trained side, affects both positive and negative learning. In the animals with posterior splits the takes of the positive were only slightly lower on the left than on the right and very much higher than in the animals with anterior splits. This difference is significant at 0·05 % (if we exclude PGF, which is quite anomalous). Evidently the 'positive' memory record, ensuring take, can be transferred (or that on the other side used) even with a split vertical lobe system.

Finally, in five out of eight animals with posterior splits the takes of 'negative' were *less* on the left than the right, whereas in anterior splits they were nearly always more. There is no special reason why they should be other than equal in the posterior

Table 3. *Animals with the median inferior frontal lobe removed*

(Training and abbreviations as Table 1. Besides showing the total amount of the median inferior frontal lobe removed, also shows the quantity removed in the mid-line, on the same scale (0–3).)

	Train right (160 trials)				Train right (80 trials)				Test left (80 trials)				Amount removed		Damage	Subfr.			
																L.		R.	
	S+	R−	%C	%A	S+	R−	%C	%A	S+	R−	%C	%A	msf.	mif.	in mid-line	dors.	ventr.	dors.	ventr.
									Smooth +										
Large removals																			
460 = PFM	78	45	71	98	38	22	70	48	37	21	70	46	1	2	2	0	0	0	0
461 = PFN	69	22	79	86	40	80	90	50	34	22	65	43	0	2	2	1	0	1	0
468 = PFO	75	64	57	94	40	32	60	50	37	38	48	46	0	3	3	1	0	1	0
469 = PFR	45	44	51	56	31	22	61	39	31	35	45	39	0	2	3	0	0	0	0
313 = PFP	74	41	71	93	35	22	66	44	31	30	51	39	0	3	3	0	0	0	0
314 = PFQ	70	45	66	80	40	38	53	50	40	40	50	50	0	3	3	0	0	0	0
n = 6	411	261	65·8	84·5	224	144	66·7	47	210	186	54·8	43·8							
Small removals																			
306 = PET	63	18	78	79	38	7	89	48	38	5	91	48	0	1	1	0	0	0	0
316 = PEW	58	12	79	73	33	4	86	41	36	3	91	45	0	1	1	0	0	0	0
n = 2	121	30	78·5	76·0	71	11	87·5	44·4	74	8	91	46·3							
									Rough +										
	R+	S−			R+	S−			R+	S−									
318 = PEY	46	12	71	58	31	18	66	39	35	17	73	44	0	2	2	0	0	0	0
467 = PEZ	47	9	74	59	37	16	76	46	37	20	71	46	0	3	3	0	0	0	0
473 = PFA	40	14	66	50	33	8	81	41	28	5	79	35	0	3	3	2	1	2	1
319 = PEF	80	73	54	100	39	31	60	49	37	36	51	46	0	2	2	0	0	0	0
320 = PEG	62	14	80	78	39	11	85	49	35	9	83	44	0	3	3	0	0	0	0
428 = PEI	58	15	77	73	31	10	76	39	33	25	60	41	0	3	3	0	1	0	0
468 = PEK	55	30	66	69	39	27	65	49	36	31	56	45	1½	3	3	0	0	0	0
n = 7	388	167	69·7	69·6	249	121	72·8	44·5	241	143	67·6	43·0							

splits, but the difference from the anterior splits emphasizes that the latter had *not* learned not to take the negative on the left and that the left side could not use the record of the right.

Removal of the median inferior frontal

After this operation six animals were trained with smooth and seven with rough positive (Table 3). The animals showed a marked preference for rough (Figs. 1, 2). Of those trained with smooth positive only two showed progressively more correct responses as training proceeded (460 = PFM and 461 = PFN, the mean for % C being 75 %); the performance of four others became *more* indiscriminate as training proceeded (468 = PFO, 469 = PFR, 313 = PFP and 314 = PFQ, the mean for % C being 61 %).

Of the seven trained with rough positive five showed a strong rough preference from the start. Two of these failed to improve in performance. A third animal (PEF) took nearly all of the objects presented to it and thus showed no preference and no improvement. The learning of these animals was, in short, strikingly slower than that of either normal or split-brain animals. A good control is provided by two octopuses in which the operation removed only a small amount of the median inferior frontal; these two learned well with smooth positive (Fig. 2, 'dummy ops').

Tests on the untrained (left) side showed some accuracy in two of the animals trained with smooth positive (Table 3), but in both of these a few intact median inferior frontal fibres were found in the mid-line. In the other four animals there was no sign of transfer and in none of them were there intact commissural fibres. The animals trained with rough positive mostly appeared to discriminate on the left, as was to be expected from the initial preference for rough.

It is clear that the median inferior frontal has a very important part in the tactile learning process. The operations had mostly been very successful in removing only this lobe, leaving the subfrontal intact (Table 3). Of course the subfrontals could not have been operating normally, however, since they were deprived of all input from above. For the present, therefore, all we can say is that interference with the inferior frontal system in this way profoundly disturbs tactile learning, reversing the preferences and delaying but not preventing the setting up of long-term memory records.

DISCUSSION

These results contain several examples of the reversal in preference that has been described elsewhere (Wells & Young, 1968). The animals with brains split, whether completely, or at the front or back, all showed a less than normal preference for smooth, sometimes producing an actual excess of takes of rough. Removal of the median inferior frontal also produced this effect. In most animals this change of preference is accompanied by an excessive tendency to take objects, including both positive and negative. The association of the two effects may of course be fortuitous, but it supports the suggestion already put forward that the rougher objects produce the greater number of signals that tend to produce a take but that this tendency is inhibited, perhaps by those parts of the system containing amacrine cells, namely the vertical and subfrontal lobes. It is presumably the reduction in the number of these cells that leads to the increased take after the operations and perhaps also to the greater tendency to take rough.

The experiments show that the commissural fibres through which tactile training on one side allows correct response on the other are mainly in the median inferior frontal lobe. No effective transfer by way of the subfrontal commissure is possible after the median lobe has been removed, and only at most a little through the vertical lobe system. Further experiments are needed to show whether the transfer through the median inferior frontal involves establishment of a representation in the memory regions of the untrained side or use (through the median inferior frontal) of that on the trained side.

When tested on the untrained side the animals with the inferior frontal system divided showed a much lower tendency to take the positive object as well as a greater tendency to take the negative than on the trained side. This is good evidence that training consists both in learning to take the one object and to reject the other. Neither form of association could form on the untrained side in the divided animals. This phenomenon did not appear after division only of the posterior (vertical lobe) region.

It is surprising that elimination of half of the brain by splitting produces so little alteration in the performance of the trained side. Perhaps this is because with the comparatively small number of animals used, small effects do not appear.

The discrimination was in any case a very simple one. As with the vertical lobe in visual learning one might expect the effect of removing a proportion of the available tissue to be most apparent in difficult discriminations. This matter has yet to be investigated with touch learning and tasks of graded difficulty.

SUMMARY

1. Octopuses with the whole supraoesophageal lobe divided in the mid-line show a lesser preference for smooth objects than normals.

2. Learning of a simple successive tactile discrimination can proceed as fast in such a half-brain as in a whole brain.

3. Animals in which the anterior part of the supraoesophageal lobe alone was split also learned approximately as fast as normals. When tested on the untrained side they showed at most slight signs of 'transfer'. The unsplit vertical lobe system apparently mediates little transfer, either of learning to take or not to take an object.

4. Animals with the posterior part of the supraoesophageal lobe alone split learned less well than normals or those with anterior splits, the deficit being due to the large number of takes of the negative object. Tests on the untrained side showed that good transfer of the capacity for positive and negative learned response occurred through the intact inferior frontal commissures.

5. Animals without the median inferior frontal lobe showed a marked preference for rough objects. Of six trained with smooth positive only two showed increasingly correct discrimination. Four out of seven animals trained with rough positive showed an increasingly correct performance as a result of training. The performance of the others got worse as training proceeded. It is not clear whether this learning deficit is due to the excessive rough preference or to the absence of some part of the learning mechanism owing to removal of the median inferior frontal lobe.

6. Tests on the untrained side of animals without median inferior frontal show no capacity to discriminate. This shows that the median inferior frontal is vital to lateral transfer and confirms that learning cannot readily be transferred through the vertical lobe system.

We are grateful to the Director and Staff of the Zoological Station at Naples for the facilities that made the work possible. The tanks and water supply were provided by a grant from the Science Research Council. The work was assisted in part by the Air Force Office of Scientific Research through the European Office of Aerospace Research, OAR, United States Air Force, under grant AF EOAR 66–53, and in part by the Science Research Council of Great Britain. We are grateful to Mr G. D. Sanders and Mr D. Gaffan for discussion of the results and assistance in preparing the MS.

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