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A subset of cingulate cortical neurones is specifically activated during alcohol-acquisition behaviour

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ABSTRACT

A new need is associated with the formation of behaviour directed at its satisfaction. In chronically ethanol-treated rabbits a bodily need develops to acquire and consume alcohol. The present study examined the firing properties of single neurones in the cingulate (limbic) cortex of chronically ethanol-treated rabbits. The main questions of this study were: are there neurones in the cingulate cortex which specifically increase their firing during alcohol-acquisition behaviour (AAB)? What is the relationship between the neuronal mechanisms of pre-existing and newly formed behaviour? Adult rabbits were taught to acquire food by pressing pedals. After 9 months of ethanol treatment, the same rabbits were taught to acquire ethanol (15% solution in a 0.5-mL capsule) by means of the same instrumental method. Activity of the 118 neurones was recorded from the cingulate cortex. The comparison of activity of each neurone in AAB and food-acquisition behaviour (FAB) enabled us to reveal that their subservings overleap substantially but not completely: 41% of 'common neurones' involved in the subserving of both FAB and AAB as well as 5% of 'alcohol-neurones' (alcohol-acquisition specific cells) were found. We think of the latter neurones as units that were specialized during the forming of alcohol-seeking behaviour. Thus, present experiments help us not only to answer the above questions but also to provide an additional insight into the nature of similarity between neuronal mechanisms of long-term memory and long-lived modifications resulting from repeated drug exposure.

Keywords alcohol, behaviour, cingulate cortex, euphoria, learning, neurone, place units.

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A new need is associated with the formation of behaviour that is directed to satisfy the need in question. A suitable model of such need established *de novo* in adults is the need of alcohol.

After a chronic ethanol treatment (CET) in rabbits a bodily need develops and the instrumental alcohol-acquisition behaviour (AAB) can be elaborated. Do the subserving of AAB and pre-morbid behaviour overlap? If they do, what are the behavioural specializations of the common and specific neurones? It is apparent that the key to these questions lies in the behavioural specialization of neurones, i.e. the functional systems to which they belong (Shvyrkov 1986, Alexandrov & Jarvilehto 1993, Alexandrov *et al.* 2000a).

In our previous studies, different types of neuronal specializations were identified in various brain areas of freely moving rabbits engaged in instrumental food-acquisition behaviour (FAB) in a cage equipped with two pedals and two feeders (Alexandrov *et al.* 1990a, b, 1991, 1993, 1994, 2000a, b). The diverse types of specializations can be classified into two main categories: 'M-' and 'L-neurones'.

'M-neurones' are activated in relation to different *movement* systems, established early in an ontogeny. Their activation is selectively related to a certain movement. 'L-neurones' are activated in relation to systems of comparatively new behavioural acts formed during an animal's *learning* of instrumental FAB in the

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experimental cage. *Unidentified* 'U-neurones' do not show consistent activation during the given task.

It has been shown previously that cingulate (limbic) cortex is one of the most sensitive structures to acute and CET, and acquires more L-neurones with new specializations after learning than did other studied brain structures (Klemm *et al.* 1976, Shvyrkov 1986, Alexandrov *et al.* 1990b, 1991, 1993, 1994, 2000a, b). In the present study, unit recordings were carried out from the area 29d of the cingulate cortex in freely moving rabbits.

The specific goal of the present study was to reveal if the mechanism for the formation of AAB was the involvement in the subserving of the AAB of the neurones of pre-morbid FAB along with the specialization of the new L-neurones specific to the AAB into 'alcohol-neurones'. The study has important implications for tackling the more general question: what is the relationship between the neuronal mechanisms of pre-existing and newly formed behaviour?

MATERIALS AND METHODS

Subjects

Five male adult rabbits (*Orictolagus cuniculus*; weight about 3 kg), preferring ethanol over water, received a nutritionally adequate diet. The animals were taken care of in accordance with the institutional guidelines (Council of Europe No. 123, Strasbourg, 1985). All efforts were made to minimize animal suffering.

Food-acquisition training

Before CET (see below) all rabbits were taught to acquire food by pressing one of the two pedals in the experimental cage (described in detail in Alexandrov *et al.* 1990b). Pressing of the pedal activated an automatic feeder on the same side of the cage. Each rabbit repeatedly carried out the food-acquisition task involving a constant series of acts (behavioural cycle: pressing the pedal, turning to the feeder and taking food from the feeder) at both sides of the cage (front and rear walls in relation to the video camera; see recording techniques).

Chronic ethanol treatment

During the 9 months of CET, the animals could freely choose between ethanol (7% first 2 weeks, 10% later) and water permanently present in water bottles (Cemic OY, Helsinki, Finland). Alcohol consumption at the end of CET was 2.9 ± 1.0 g kg^{-1} a day.

After the CET, the same rabbits were taught to acquire alcohol by means of the same instrumental method in the same experimental cage. As taking solid and liquid substances is very different, we presented ethanol solution in 0.5-mL gelatinous capsules to make the final acts of FAB and AAB more similar. It was shown that the highest ethanol intake during operant behaviour in alcohol-preferring rats takes place at the 15% concentration (Murphy *et al.* 1989). We have found similar results in pilot experiments with rabbits. Hence, we filled capsules with 15% ethanol solution during the recording experiments. After the training, unit recording began.

Recording techniques

Electrophysiological and behavioural recording techniques, as well as the criteria for the classification of the behavioural specialization of the units have been described in detail elsewhere (Alexandrov *et al.* 1990a, b, 1991, 1993, 2000a, Gorkin & Shevchenko 1991).

Unit activity was recorded from the area 29d of the cingulate cortex ($P = 11.1 \pm 0.3$; $L = 3.3 \pm 0.1$, according to Vogt *et al.* 1986). Glass microelectrodes with 2.5 m KCl, tips of 1–3 μm diameter and impedance of 1–5 MΩ at 1.5 kHz were used and driven by a micromanipulator. During the recording of the activity of each neurone, a rabbit performed alternating series of both food and alcohol-acquisition instrumental behavioural acts.

Unit activity, EMG (*m. masseter pars profundus*) and actographic marks of the behaviour (see Alexandrov *et al.* 1990b) were tape-recorded. In addition, the animal's movement from the pedal to the feeder, or vice versa, was recorded by a photocell, fixed to the head of the animal, which responded to photodiodes located in the middle of the front and rear walls of the cage (left and right behavioural cycles, correspondingly in relation to experimenter position) between the pedal and the feeder. The rabbit's behaviour was video-recorded with the unit activity (audio-channel), the light indicators of the pedal pressing and head lowering, the counters of the cumulative number of spikes, and of time.

The depth of each active unit's location, encountered during microelectrode penetration was measured by means of a potentiometer attached to a micromanipulator and connected with a calibrated scale showing the vertical location of the recording tip.

Data analysis

Each behavioural cycle on the left side of the cage was divided in accordance with the behavioural actographic marks into five stages (behavioural acts): (1) turning a head to a pedal; (2) approaching a pedal; (3) pressing a pedal; (4) approaching a feeder and (5) seizing food or

capsule in a feeder. Behavioural cycle on the right side of the cage was divided into analogous stages (acts 6–10; see Fig. 1c). The following indices were selected as the characteristics of the activity of the neurone: the average frequency of spike activity in a particular act and the probability of an activation in the act. The average frequency of activity for the entire recording was calculated for each neurone. The exceeding by the frequency of the activity in one or several acts of the average frequency of the activity of a neurone over the whole period of its recording, by not less than a factor of 1.5 was taken to indicate activation. A neurone was considered to be specialized relative to a system of specific behavioural act if the activation in this act was observed in *all* cases (100%).

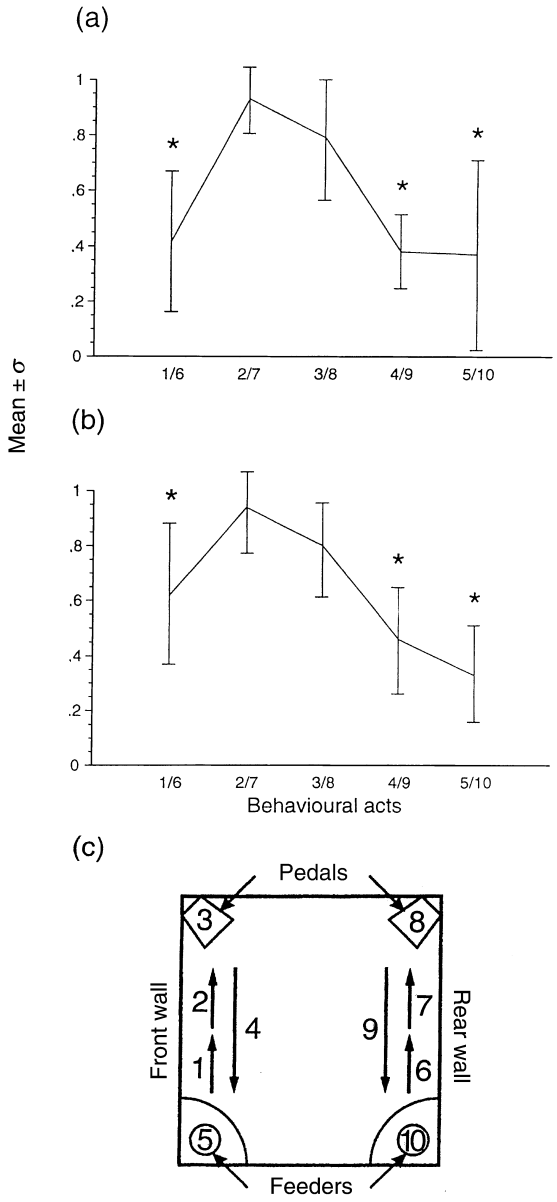

Figure 1 Collective patterns of 'non-specific' activity of eight U-neurones in the behavioural cycle on the left side of the cage: acts 1–5; and the behavioural cycle on the right side of the cage: acts 6–10 (see (c) and Materials and methods for the list of acts). Along the abscissa: numeric labels of the corresponding behavioural acts in FAB (a) and AAB (b). Activity of different U-neurones appearing in the same behavioural acts, but belonging to different behavioural cycles (e.g. 1 and 6, 2 and 7 and so on) was counted together. Along the ordinate: 'non-specific' activity, normalized in respect to its maximum for eight U-neurones (*$P < 0.02$ to < 0.001, *t*-test). See text for more details.

The corresponding numbers of acts were indicated along the abscissa in all of the graphs, representing the patterns of the activity of neurones in behavioural cycles. The mean frequency of the activity of the neurone in a given act normed relative to the frequency of the activity in the specific act was given along the ordinate (see figures). Graphs were used to assess the activity of the neurone in each act of the behaviour being studied over the course of the entire period of recording and to determine its specialization were plotted for all of the neurones analysed. The significance of the differences of the unit activity in the acts was determined on the basis of the Student's *t*-test (significance limit $P = 0.05$) through comparison of the mean frequencies of the activity for each pair of acts.

The units were divided into two groups: unidentified (do not show consistent activation during the behavioural cycles of instrumental behaviour – the U-neurones) and specialized in relation to the systems of behaviours under study (activated in constant relation to a certain stage of the repeated behavioural cycle). The latter group was further divided into two groups with different behavioural specialization: M- and L-neurones (see Introduction). Neurones that showed activation in relation to a particular movement of the body, head or lower jaw were considered to be specialized relative to the systems formed earlier in ontogeny (Shvyrkov 1986, Alexandrov *et al.* 2000a). Whether their activation appears or not was related specifically to a certain movement but independent of its behavioural context. Activation appeared during the same movement in different behaviours, e.g. turning to the right when approaching the feeder on one side of the cage or approaching the pedal on the opposite side of the cage (M-neurones). L-neurones showed activation in relation to novel behavioural acts established late in individual development, such as during animal's learning in the experimental cage (e.g. approaching the feeder, approaching the pedal, pressing the pedal). Whether their activation appears or not was specifically related to a certain behavioural act but independent of its motor characteristics. Similar activity was elicited when the animal pressed the pedal with the left paw, right paw or both. Many of the L-neurones became active only when the animal pressed a certain pedal, say in rear-wall but not in front-wall behavioural cycle. In the activity of such neurones a 'specific' phase may be distinguished –

expressed activation; it appears during that behavioural act, in relation to a system of which these neurones were specialized. This activation usually greatly exceeds the 'non-specific' activity of this neurone recorded during other behavioural acts; furthermore, 'non-specific' activity is more variable and appears not in 100%. The behavioural specialization of a neurone is its permanent characteristic (see Discussion). That is why neuronal activity can serve as an index for the actualization of a specific system, and the 'non-specific' activity of a neurone may indicate the specific system's retrieval from memory during performance of other behavioural acts.

During the recording period, alcohol was left in the home cage in the amount which, added to the volume, consumed during the previous-day experiment, equalled the average day consumption.

The statistical significance of the differences between the number of units belonging to the different groups was estimated by the χ^2 and Fisher's exact tests (significance limit $P = 0.05$). The duration of each behavioural act of AAB and FAB was determined and compared by Student's *t*-test (significance limit $P = 0.05$).

Histology

After the experiments, the rabbits were sacrificed with an overdose of nembutal, the brains were fixed in 10% formalin solution and dehydrated by increasing concentration of ethanol. Serial frontal slices were prepared (thickness 10–20 μm) and every 10th section was stained by means of the Nissl method. The thickness of the II–IV cortical layers (containing small, densely packed cells) and V–VI layers (containing large, mostly pyramidal neurones) was determined. The location of the units in the upper and lower layers was determined on the basis of calibrated scale readings (see above) and morphological analysis.

RESULTS

Behavioural data

It was evident from the observation that animals were not attracted to the capsules per se. Animals did not consume empty capsules. Usually an animal stopped AAB (i.e. refused to take capsules with ethanol from the feeder presented to it after pedal pressing) earlier than it stopped FAB. However, rabbits that had just refused ethanol capsules, willingly drank considerable amounts of ethanol from the syringe presented to them by an experimenter. Daily alcohol consumption during a recording session did not exceed 0.40 g kg^{-1}.

When the duration of each behavioural act of AAB was compared with that of FAB, it was apparent that most acts of the former behaviour were significantly slower than that of the latter (see Table 1).

Neuronal activity

Common neurones

Out of 118 units which activity was recorded during behavioural cycles of both behaviours, 63 (54%) were U-neurones. It is obvious from the above that U-neurones had no consistent ('specific') activation in any acts of FAB and AAB. However, some U-neurones demonstrated increase in their discharge frequency during certain realization of some act. For this group of U-neurones, characteristics of the activity in FAB and AAB were compared. Analysis of their activity involved normalization of the frequency of activity with respect to the maximal frequency of activity observed during a certain act. Figure 1 shows the distribution of means of normalized frequencies in eight U-neurones having prominent increase in activity rate during the approach and/or pressing the pedal in comparison with the rate during other acts. The figure demonstrates that the pattern for the distribution of U-neurones activity rates in FAB (Fig. 1a) was equal to that in AAB (Fig. 1b). In both cases, the frequency in acts 2/7 and 3/8 was significantly higher than in acts 1/6, 4/9 or 5/10. No significant differences were found between the same acts in FAB vs. AAB.

Activation of 25 (21%) neurones were selectively related to a particular movement. These units were classified as M-neurones (see above). All M-neurones demonstrated similar activity in FAB and AAB, i.e. if a neurone had activation in FAB during certain movement, e.g. turning to the right, this neurone also activated during turning to the right in AAB.

Table 1 Duration of acts of FAB and AAB (n = 61–71)

	Duration of acts: $m \pm \sigma$ (s)									
	N1	N2*	N3*	N4*	N5	N6	N7*	N8*	N9*	N10
FAB	0.7 ± 0.6	0.7 ± 0.4	0.7 ± 0.4	0.6 ± 0.3	1.1 ± 0.4	1.1 ± 0.8	0.6 ± 0.4	1.0 ± 0.5	0.5 ± 0.2	1.5 ± 0.7
AAB	0.7 ± 0.7	1.0 ± 0.8	1.1 ± 0.7	0.8 ± 0.3	1.2 ± 0.5	1.3 ± 0.9	1.1 ± 1.0	1.5 ± 1.0	0.8 ± 1.0	1.4 ± 0.6

Left behavioural cycle: NN 1–5, right behavioural cycle: NN 6 –10 (see Materials and methods for the list of acts).
* Significantly different values (two-tailed *t*-test; $P < 0.01$).

Table 2 Number of units in groups of L-, M- and U-neurones

	Number of neurones associated with behaviours		
Group of specialization	AAB + FAB	AAB alone	FAB alone
L-neurones 30/118	24 (20%)	6 (5%)	0
M-neurones 25/118	25 (21%)	0	0
U-neurones 63/118 (54%)			

Activation of 24 (20%) cells fitted all the criteria for the L-neurones in both FAB and AAB. We have observed earlier that relative numbers of limbic cortex M- and L-neurones were not significantly different in chronically (9 months) alcohol-treated rabbits after acute ethanol administration (Alexandrov *et al.* 2000b). Conversely, there were more limbic L- than M-neurones in healthy (not exposed to CET) rabbits: 28 and 17% correspondingly (Alexandrov *et al.* 1990a).

Hence, 41% of all cells examined were 'common neurones', i.e. neurones involved in the subserving of both FAB and AAB (see Table 2).

Nine L-neurones were activated during the approach to the pedal and/or pressing the pedal or during the approach to the feeder and/or the item seizure in FAB; these neurones were activated in the same acts in AAB (Fig. 2).

Six neurones, activated only when a rabbit was located in a certain place with varying behavioural context (instrumental behaviour, placement of the animal into a certain place by the experimenter, orienting behaviour, grooming, etc.) were classified as place units (cf. O'Keefe 1976). All six units demonstrated 'specific' activation during some act of FAB. These place units had activation in the same place 'fields' in FAB and AAB. However, one place neurone having its 'field' near one feeder and showing activation during the approach to the feeder in FAB, demonstrated in AAB activation not only during the approach to the feeder but during the opposite motion: away from the feeder. Thus, it becomes apparent that this place neurone lost the 'directionality' of its activation in the AAB.

In parallel with the similarities of neuronal subserving of FAB and AAB, we detected differences between the quantitative characteristics of L-neurone activity in FAB and AAB as well. The frequency of a 'specific' activation of any L-neurone in FAB was confronted with the frequency of a 'specific' activation of the same neurone in AAB. It was shown that the frequency of discharges of some units in animals performing AAB decreased progressively as the alcohol intake increased (Woodward *et al.* 1998). Thus, we proposed that the re-distribution of food- and alcohol-related activity levels as a function of consumption of alcohol might take place. Based on the above proposition, we divided all L-neurones into two groups. The activation of the first group ($n = 14$) was recorded till the animals consumed an ethanol dose of 0.15 g kg^{-1}; the second ($n = 10$) with the increase of the dose throughout the daily recording session. There were significantly more neurones in the second group which had a greater activation in FAB than in AAB (sign criteria, $P = 0.05$). There were equal number of neurones with larger and smaller activation in FAB in the first group.

Six out of 24 L-neurones showed significantly different frequency of 'non-specific' activation in AAB when compared with FAB. Two neurones gave larger activation in FAB, whereas four units discharged with higher frequency in AAB. Figure 3 demonstrates the 'non-specific' activation of the neurone that had a 'place field' near the rear wall of the cage between the right pedal and feeder and gave 'specific' activation during acts occurring in the 'field'. The 'non-specific' activation of this neurone appearing during the approach to the left pedal both in AAB and in FAB was significantly more prominent in the latter behaviour.

Alcohol-neurones

Six (5%) out of 118 neurones had 'specific' activation (i.e. activation appearing in all realizations of behaviour) in AAB. In FAB they gave only 'non-specific' activation. These units were classified as specialized in relation to the acts of AAB, i.e. as 'alcohol-acquisition specific' cells – 'alcohol-neurones' according to the criteria given in the Introduction and Materials and methods. 'Food-acquisition specific' cells were not found. Thus, if we consider only FAB, we should increase the number of the U-neurones by 5%.

Two 'alcohol-neurones' gave 'specific' activation during the animal's approach to the feeder and/or seizing the capsules. Four neurones were specifically activated during the approach and/or pressing the pedal. One neurone out of the latter set is represented in Fig. 4.

We have not revealed preferential localization neither of the specific 'alcohol-neurones' nor of the 'common neurones' in the upper or lower layers of the cortex.

DISCUSSION

In spite of a small amount of ethanol consumed during a recording session, we have not noticed any physical signs of abstinence in rabbits. On the one hand, these

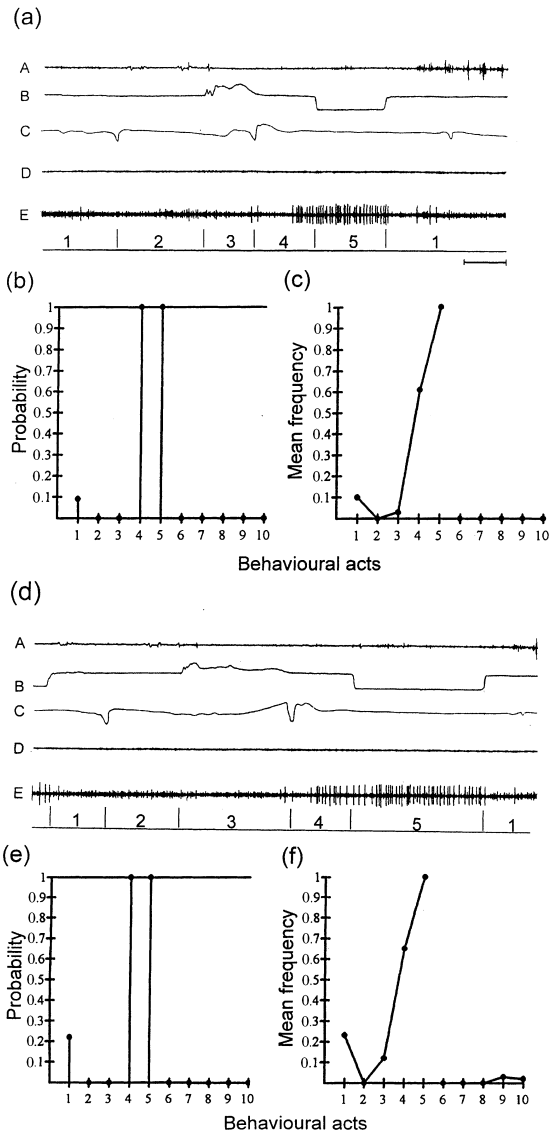

Figure 2 Example of the activity of the L-neurone that was similarly activated during FAB and AAB. Neurone had 'specific' activation during approaching the left feeder (act 4) and the item seizure inside the feeder (act 5) both in FAB and AAB. Above (a, d): examples of the neuronal activity in the left behavioural cycle of FAB (a) and AAB (d), single realization. (A) EMG of *m. masseter*, (B) actogram of behaviour in the left cycle (upward deflection = pedal pressing, downward deflection = lowering head into feeder), (C) actogram showing the position of the head in relation to either wall (left or right) of the cage between the pedal and the feeder (downward deflection = head is near middle of the wall; see Materials and methods), (D) actogram of behaviour in the right cycle, (E) neurone activity. 1, 2, 3, 4, 5 – intervals that correspond with the behavioural acts 1, 2, 3, 4, 5 of the left cycle (see Materials and methods for the list of acts). Horizontal bar on the right = 200 ms. Below: left graphs (b, e) – along the abscissa: the numeric labels of the corresponding behavioural acts; along the ordinate: the probability of the presence of activation in the corresponding acts. Note that activations of the neurone were observed in all realizations. Right graphs (c, f) – along the abscissa: the numbers of acts; along the ordinate: the normed average frequency of activity. Note the difference between the frequency of the 'specific' activations and activity in the other acts.

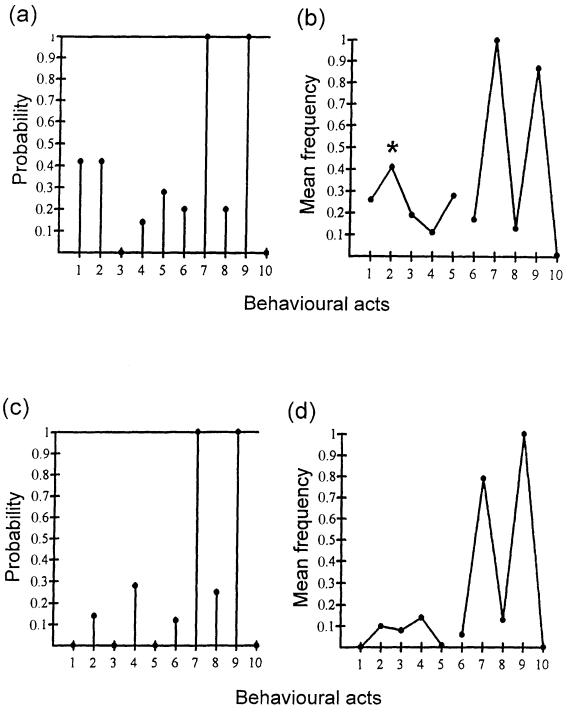

Figure 3 Difference in the frequency of 'non-specific' activations in the neurone having similar 'specific' activations. All graphs as in Fig. 2. These graphs show that the neurone had 'specific' activations during approaching the right pedal (act 7) and approaching the right feeder (act 9), i.e. when rabbit passed through the 'place field' of the neurone (see text). These activations were similar in food (a, b) and alcohol acquisition (c, d) behaviour. However, 'non-specific' activation appearing during approaching the left pedal activation was more prominent in FAB (*$P < 0.05$, *t*-test).

signs can rarely be observed in animals voluntarily administering the drug and are not necessarily indicators of dependence (Koob *et al.* 1989). On the other hand, it was shown that adulteration of alcohol solutions by an additive with an aversive taste is not sufficient to suppress alcohol taking in alcohol dependent animals, although they tend to reduce their consumption (Wolffgramm 1991). Wolffgramm considers the persistence of alcohol taking behaviour in spite of adulteration as a strong argument for the presence of behavioural dependence that may be equivalent to drug addiction in men. Our behavioural data show that the rabbits did not eat empty capsules but drank ethanol from the syringe right after rejecting capsules filled with ethanol. In this connection, we can consider capsules in our experimental conditions as a peculiar additive with an aversive taste and propose that the dependence and need of alcohol were established in our rabbits after 9 months of CET.

The results of the comparison of activity of cingulate neurones in FAB and AAB indicate that their subservings overlap substantially. Out of all recorded cells, 41% are 'common (L- and M-) neurones' involved in the subserving of both FAB and AAB.

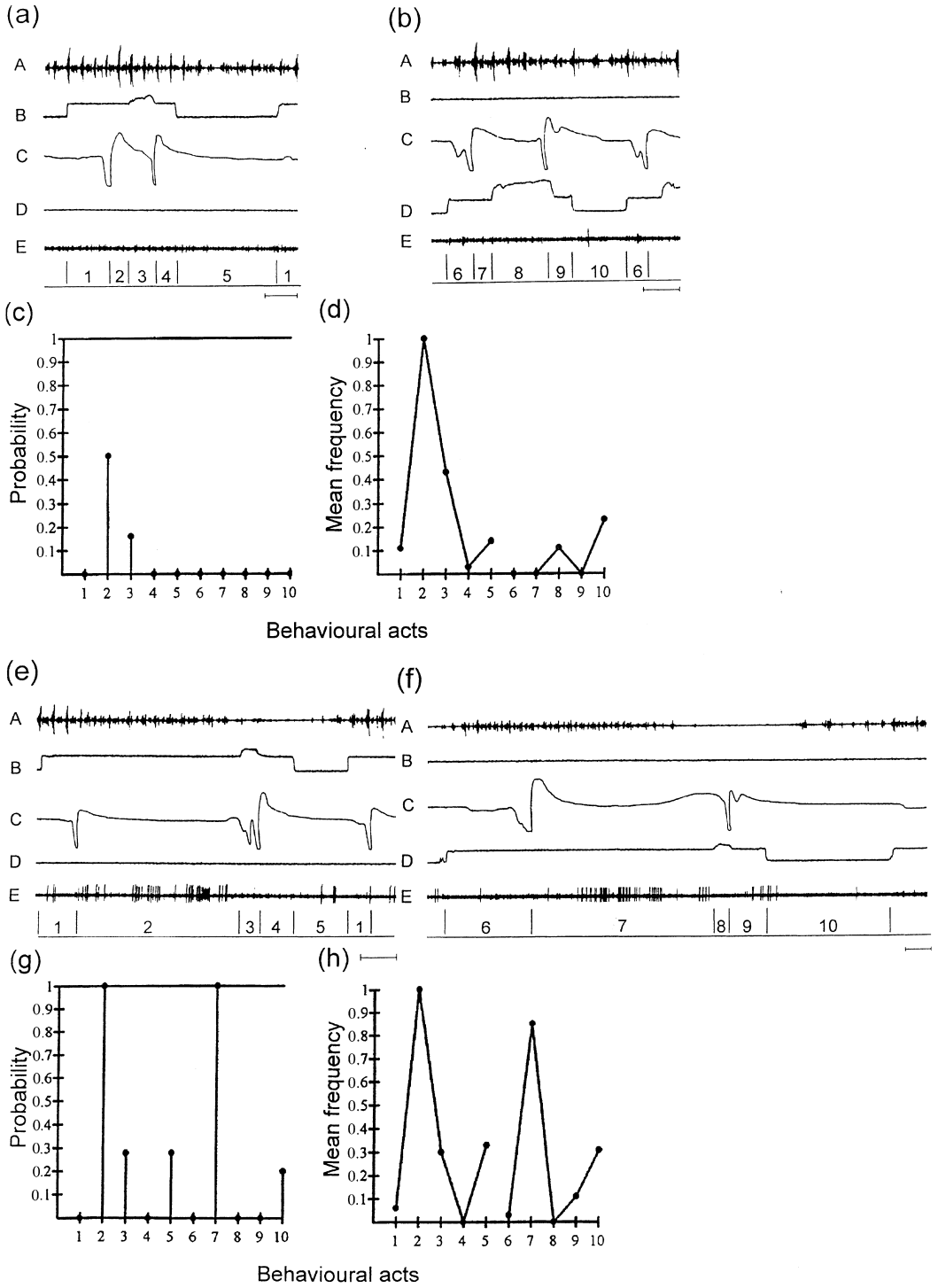

Figure 4 Example of the activity of the L-neurone that had 'specific' activation only in AAB. This neurone had 'specific' activation during approaching both pedals: the left (e, act 2) and the right (f, act 7) only in AAB (e–h). In FAB (a–d), activation of this neurone was 'non-specific'. Above: examples of the compared neuronal activity in the left (a, e), and right (b, f) behavioural cycles of behaviours, single realizations. (A–E) – as in Fig. 2. Horizontal bar on the right = 200 ms. Below (c, d and g, h): graphs as in Fig. 2. Note that activations of the neurone were observed in all realizations of acts 2 and 7 in AAB, but not in FAB.

Present data showing substantial overlap of subservings of FAB and AAB are strong experimental evidence in favour of an assumption (Seth 1998) that the coherence between subservings of different behaviours but not internal arbitration between them is the right way to consider the action selection problem.

As to the 'common neurones', the U-neurones must be considered. Our earlier data indicate that the U-neurones are cells related to some systems of 'other behaviours', not analysed in the given study (Alexandrov *et al.* 1990a, 1993). There are also data in literature indicating that even much variable discharges are not 'neural noise' but indicators of the neurone's involvement in the organization of behaviour (Vaadia *et al.* 1995, Ferster 1996). In connection with this data there is a good reason to believe that U-neurones (representatives of 'other behaviours') make a contribution (very similar) to subserving both FAB and AAB.

Studies in our laboratory supported the suggestion that the mechanism of the formation of behaviour (learning) is the recruitment (specialization) of 'new' (previously silent) neurones into the subserving of specific new behaviours (Shvyrkov 1986, Gorkin 1988, Alexandrov & Jarvilehto 1993, Alexandrov *et al.* 2000a). Data from other laboratories (Thompson & Best 1990, Wilson & McNaughton 1993, Chang *et al.* 1994, Swadlow & Hicks 1997, Woodward *et al.* 1998, Jog *et al.* 1999) confirms the suggestion that learning involves 'new' neurones rather than 're-learning' (the replacement of pre-existing specialization by a new one) of the 'old' cells, that newly formed unit specialization remains the same during the whole period of the recording (weeks and even months), and that there are many silent neurones in different brain areas that may become active at some time. Taking the behavioural specialization of a neurone to be its permanent characteristic, we can consider the data for 'common neurones' as an evidence in favour of the assumption that neuronal mechanisms of pre-existing behaviour provide the basis for the formation of neural mechanisms of a new behaviour (AAB) directed at the satisfaction of the need of alcohol established *de novo*. 'Common neurones' specialized in relation to systems of pre-existing behaviour do not lose their pre-morbid specialization but undergo certain modifications. The modifications are that elements of pre-morbid memory (systems in relation to which 'common neurones' belong) start to subserve the realizations of not only pre-existing but also the newly formed behaviour.

It was proposed in psychology that the need of alcohol formed *de novo* does not simply 'add' to the pre-morbid needs, but modifies their structure. Different pre-existing actions formed in the frame of pre-morbid motives transform and begin to serve as a means to satisfy the need of alcohol (Bratus 1974). It is conceivable that our data are consistent with this proposition.

Our data show that participation of the cingulate M-neurones and some of the U-neurones is considerably similar in the subserving of behaviour directed at the satisfaction of different needs in the similar environment by means of similar movements. Differences in neuronal organization of FAB and AAB were defined by examining the activity of L-neurones. Although many of the L-neurones belonged to the group of the 'common neurones', the quantitative characteristics of their 'specific' and 'non-specific' activation were different in the compared behaviours. It means that the same systems (in relation to which these L-neurones are specialized) are differently involved in the FAB and the AAB.

As it was shown, animals stopped the AAB earlier than FAB. Most likely their need of capsules filled with ethanol was satisfied earlier than of food. In this connection of particular interest is the fact that after the consumption of a certain amount of ethanol, neurones of larger activation in FAB than in AAB became predominant within the population of the 'common L-neurones'. This fact suggests that at least one factor determining the mentioned variety of activation's characteristics in the same unit in different behaviours is the magnitude of the need that is being satisfied during realization of a given behaviour. Supporting this argument is the work of Kendrick & Baldwin (1989) who showed that the magnitude of the neuronal activation of zona incerta cells to the sight of the salt solution diminished over time of consumption of $NAHCO_3$ solution and that to food increased so that food become a more 'potent stimulus'.

Subservings of FAB and AAB overlap substantially but not completely: 5% of the 'alcohol-neurones' having 'specific' activation only in AAB were found. It was shown in monkeys and rats that mechanisms subserving juice- and cocaine-acquisition behaviour 'are at least partially separable at the neuronal level' (Bowman *et al.* 1996, Carelli & Deadwyler 1997, p. 1072). Our results, showing the existence of the specific 'alcohol neurones' testify that similar regularity is operating to organize FAB and AAB. Assuming that learning involves specialization of new neurones, we regard the 'alcohol neurones' as cells that were specialized during the forming of alcohol-seeking behaviour.

Neural activity during behaviour subserves the achievement of the result of a functional system for which a particular neurone is specialized; when the result of a given behavioural act is achieved, the activity of the neurone ceases (Shvyrkov 1980, Alexandrov 1999). Based on the study of acute ethanol effect on the activity of the limbic cortex neurones in healthy rabbits (Alexandrov *et al.* 1990a), we concluded that acute ethanol administration has an effect on the L-neurones that is comparable with the effect of achieving the result mediated by the systems to which these L-neurones belonged, i.e. alcohol results in the cessation of the activity in many units. It means that in healthy animals there are two ways to 'satisfaction': to achieve the result of some behaviour or to consume alcohol.

Thus, alcohol can simulate on the neuronal level simultaneous achievement of results of many behaviours. It can be assumed that euphoria is an important subjective manifestation of this effect.

The development of alcohol dependence is related to the process of specialization of specific 'alcohol neurones'. These neurones may be considered as the newer subset of a cell group 'that directs the execution of the alcohol-seeking…behavioural patterns' and 'grows into domineering "hypernetwork"…suppressing…other neurones' (Ludvig *et al.* 1998, p. 48). What are the distinct features of these neurones if one compares them with specialized neurones in healthy animals? It may be proposed that for some 'alcohol neurones' the one and only pre-requisite to the cessation of their activity exists: consumption of alcohol and the appearance of ethanol (and the substances arising as a result of its turnover) in the internal milieu.

It is appropriate to consider the differences in neuronal organization of FAB and AAB in terms of the place neurones concept. Our earlier and present data indicate that the activation of place units corresponded to the space which was divided into 'fields' in relation to the behavioural acts the animal realized while approaching the goal-objects in the given environment (Alexandrov *et al.* 1993). There are also data in literature that lend credence to this view (Breese *et al.* 1989, Winer *et al.* 1989, Winer 1996, Kobayashi *et al.* 1997). If we accept this view, all L-neurones may be considered as place units, which are active in a given place (or places), because this place was related to the appropriate results of the behaviour. The difference between those L-neurones which were in our study classified as place units and the rest of the L-neurones would then be that the latter ones belong to systems involved in the instrumental behaviours, but the first ones also to other acts realized in the environment of the experiment. Taking into account the involvement of new 'alcohol L-neurones' during learning of the AAB and differences between the activity of 'common neurones' in the AAB and the FAB, we can state that new learning in the given environment leads to its 're-division', even when the location of goal-objects is invariable; these objects acquire additional meaning. Thus, in our experiments 're-divison' implies that physically the same environment is presented by different brain activity before and after AAB formation.

We have found AAB- but not FAB-specific cingulate L-neurones. However, we cannot claim non-existence of the latter group; rather it would be more proper to say that the occurrence of revealing of 'alcohol L-neurones' in the present experiments was significantly higher than that of 'food L-neurones' (Fisher's exact test, $P < 0.05$). Indeed, the existence of specific 'food-neurones' was demonstrated in our own earlier work, although in a different study set up devoted to the comparison of neuronal sets subserving seizure of food and non-food objects (Alexandrov & Korpusova 1987) and in cited works of Bowman *et al.* (1996) and Kendrick & Baldwin (1989). We previously showed that the number of active L-neurones involved in FAB increased in alcoholic rabbits after acute ethanol administration of dose 1 g kg^{-1} (Alexandrov *et al.* 2000b). Hence, one might expect that the number of revealed 'food L-neurones' may grow with the increase of consumed amount of alcohol.

There are molecular evidences that the cyclic AMP response element-binding protein (CREB) and immediate-early genes are important components of the switch from short-term to long-term memory (Milner *et al.* 1998, Anokhin 2000). Also features of these genes activity have been found in a variety of long-term adaptive changes in brain, such as alcohol abuse, to convert short-term modifications in healthy individuals – social usage to a long-term change – addiction (Nestler & Aghajanian 1997, Robbins & Everitt 1999). It was concluded that there is indeed similarity between neuronal mechanisms of long-term memory and 'long-lived adaptations' resulting from repeated drug exposure and that the major goal of current research is to gain insight into the molecular and cellular basis of such adaptations (Nestler & Aghajanian 1997, Robbins & Everitt 1999).

There are two important components of brain reorganization in consequence with chronic alcohol use. Firstly, the toxic effect of ethanol. Some cells die and may exhibit loss of synapses preceding death, while other neurones become hyper-innervated (King *et al.* 1988). It is known that the change in the number of synapses is also a feature of structural changes accompanying memory storage (Bailey & Kandel 1993). Secondly, on the basis of the present results, we can propose that 'long-lived adaptations' in consequence with chronic alcohol use include kind of modifications not simply similar but identical to the underling long-term memory formation. In our experimental situation, these modifications are the closely connected processes of the specialization of 'alcohol neurones' and the modifications of pre-morbid (pre-existing) behaviour neurones (process of accommodative re-consolidation). The above proposition is in line with the hypothesis that drug addiction is a form of learning (Robbins & Everitt 1999).

Nestler & Aghajanian (1997) formulate a challenge in the addiction field: the problem of long-lived forms of sensitization as well as the high risk for relapse seen after months and even years of abstinence. In response to this challenge, we can propose that specialization of neurones underling the formation of new behaviours during years of abstinence adds to but not replaces permanent pre-existing specialization of specific 'alcohol neurones'.

Thus, the present experiments help us not only to answer the question of the relationship between the neuronal mechanisms of pre-existing and newly formed behaviours, but also to provide an additional insight into the nature of similarity between neuronal mechanisms of long-term memory and 'long-lived adaptations' resulting from chronic drug usage.

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