circumstances could lead to production of substantial quantities of CH_3Cl and must therefore be considered alongside other postulated sources of atmospheric CH_3Cl such as combustion of vegetation[10] and reaction of iodomethane with Cl^- in sea water[11]. That such a biological source may be a major contributor to atmospheric CH_3Cl levels is of considerable interest in view of the importance ascribed to CH_3Cl and other man-made halocarbons in controlling the levels of stratospheric ozone[1,12]. Quite apart from the environmental significance, the enzymatic incorporation of halide ion apparently directly into a one-carbon compound deserves attention as it must involve a mechanism quite distinct from the haloperoxidase-mediated halide ion incorporation previously regarded as the major route to organohalogen compounds in nature[13]. The biotechnological potential of this novel system warrants investigation; indeed, a role for the fungal gene involved can be envisaged in the development of halotolerant crop plants, although environmental considerations may limit such an application.

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Use of numbers by a chimpanzee

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Recent studies have examined linguistic abilities in apes[1–6]. However, although human mathematical abilities seem to be derived from the same foundation as those in language, we have little evidence for mathematical abilities in apes (but for exceptions see refs 7–10). In the present study, a 5-yr-old female chimpanzee (*Pan troglodytes*), 'Ai', was trained to use Arabic numerals to name the number of items in a display. Ai mastered numerical naming from one to six and was able to name the number, colour and object of 300 types of samples. Although no particular sequence of describing samples was required, the chimpanzee favoured two sequences (colour/object/number and object/colour/number). The present study demonstrates that the chimpanzee was able to describe the three attributes of the sample items and spontaneously organized the 'word order'.

Before the present study[11,12,14], Ai had been trained to name 14 objects and 11 colours by choosing among the set of symbols shown in Fig. 1. For the first experiment, five objects and five colours were selected from Ai's 'vocabulary' to form groups of sample items to be identified. The experimenter presented the sample items (for example, three red pencils) in the display

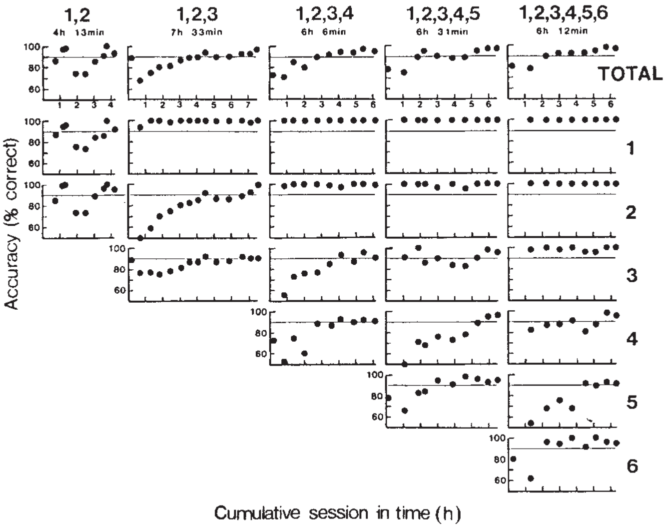

Fig. 2 Acquisition of number names for the training item, red pencils. The top row represents overall accuracy, the other rows accuracy for individual numbers. Each dot shows the performance in each session, with percentage correct plotted against the time elapsed since the beginning of training on each number set. In the first session of each stage, except for training on the number names 1 and 2, the chimpanzee received intensive training limited to the newly introduced number.

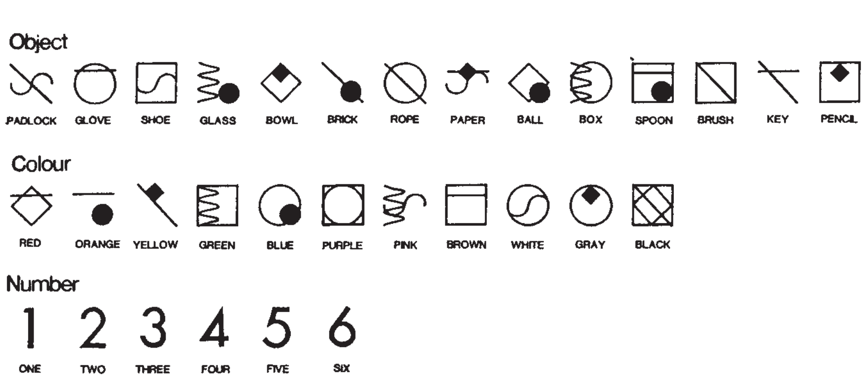

Fig. 1 Symbols used in these studies. In the symbolic matching-to-sample task, the chimpanzee was trained to identify the sample item that appeared in the display window (24 × 17 × 24 cm) by pressing an illuminated key (2 × 2.5 cm) showing the assigned symbol. These symbols could appear in various positions in a 5 × 6 key matrix on a console located below the display window. This console was interfaced with a PDP11/V03 minicomputer. The set of operative-illuminated keys was changed from trial to trial and the positions of the keys were also changed from session to session so that the symbol, rather than the position of the correct key, had to be learned. The trial ended when the chimpanzee pressed a blank key to the right of the matrix on the console. In numerical naming, two methods of displaying the sample items were used to minimize the possibility that the chimpanzee would associate each numeral with a particular display pattern. On some trials the samples were laid out in a row. The position and the spatial density of items were changed within the area of the window in each trial so that area covered was not correlated with number of items. On other trials the items were held in one hand in front of the display window. The orientation and the angular separation of items was varied from trial to trial. Depending on type of object, either or both methods were used. In the first experiment, five objects (pencil, paper, brick, spoon and tooth-brush) of five colours (red, green, blue, yellow and black) were used to form groups of items to be identified.

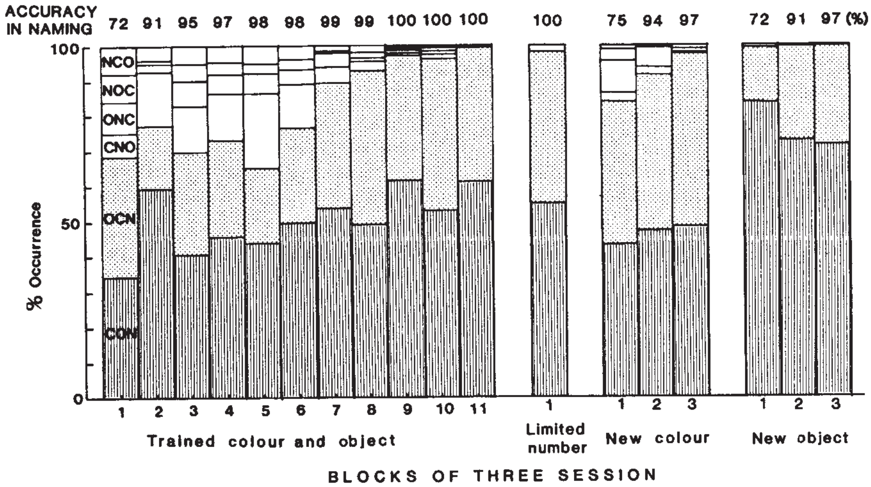

Fig. 3 Percentage of six possible sequences in three-term naming. N, number; C, colour; O, object. For example, NCO refers to trials in which the sequence 'number/colour/object' was used correctly. The open bars show the percentage of trials on which number was the first or second attribute named. The percentage of trials on which all three attributes of the sample items were named correctly is shown at the top of each column. The left panel shows the change of use of the six possible sequences as a function of three-term-naming training. A session ended when 100 rewards had been delivered. Each reward required two consecutive correct responses. Each block consists of three consecutive sessions. In the final block of three sessions of the task, the chimpanzee received 604 problems during 127 min and made only three mistakes. To investigate the determinant of the particular sequences, three kinds of tests were done. In the first test ('limited number'), the number of sample items was not varied but restricted to one of the five alternatives within a session. The objects and colours used in this first test were the same as those used in previous training. In the second test ('new colour'), new colours were introduced. The sample items consisted of the numbers from one to five, the five trained objects and five new colours, pink, brown, purple, white and grey. In the third test ('new object'), new objects were introduced. The sample items consisted of the five trained colours and five new objects, padlock, glove, glass, ball and key. In this test, the number of items to be named was restricted to one and two. Although those new samples had never been used in three-term naming, or even in simple numerical naming, the chimpanzee correctly described the three attributes of the samples. Decreasing the difficulty of numerical naming by using the same number of items in all samples in a given session, or increasing the difficulty of object or colour naming by introducing new colours and objects, had little effect on the favoured sequences. The chimpanzee seemed to have established the order of describing the three attributes of the sample items. However, the introduction of new samples had a temporary impact on the ratio of these two sequences. The introduction of new colours increased one sequence, object/colour/number. The introduction of new objects increased the use of the other sequence, colour/object/number. As those new samples were presented repeatedly, the ratio of the two favoured sequences gradually returned to the original level.

window and then pressed a start switch to light a set of numeric keys on a console. Correct and incorrect key choices were followed by different sounds. A piece of apple or a raisin was delivered after a number of consecutive correct responses. A session ended when 100 such rewards had been delivered or when 60 min had elapsed, whichever came first. Ai received one or two sessions a day. On the average, 303 trials were given in a 43-min session, with trials spaced ~8.5 s apart.

At the beginning of the first stage of training, only two numbered keys were available and only one or two red pencils were shown. The number of red pencils and corresponding number keys was increased successively. Training was continued on each number set until accuracy exceeded 90% in at least two consecutive sessions. Acquisition of number naming with red pencils only as exemplars is shown in Fig. 2. The introduction of a new number was always accompanied by poorer identification of the maximum number learned in the previous stage. For example, in the course of initial training to name six items in addition to 1, 2, 3, 4 and 5, Ai made 274 errors out of 3,004 trials, 63.1% of these confusion between 5 and 6. Moreover,

Table 1 Generalization of numerical naming to a new colour, a new object or both a new colour and object in a chimpanzee

	Accuracy on probe trials Samples				
Numbers	New colour, trained object	Trained colour, new object	New colour, new object	Accuracy by chance	Accuracy of trained samples on probe trials
1, 2	0.83 (5/6) Blue Pencil	0.50 (3/6) Red Paper	0.50 (3/6) Blue Paper	0.50	0.97
1, 2, 3	0.60 (18/30)* Yellow Pencil/paper	0.33 (10/30) Red/blue Brick	0.33 (5/15) Yellow Brick	0.33	0.99
1, 2, 3, 4	0.71 (34/48)‡ Black Pencil/paper/brick	0.54 (26/48)† Red/blue/yellow Spoon	0.56 (9/16)* Black Spoon	0.25	0.99
1, 2, 3, 4, 5	0.79 (79/100)‡ Green Pencil/paper/brick/spoon	0.57 (57/100)‡ Red/blue/yellow/black Tooth-brush	0.56 (14/25)* Green Tooth-brush	0.20	0.98

The generalization was tested by inserting probe trials in which the new colour, new object or both, was used to illustrate the number. These probe trials were included among trials during which trained samples were used. On trials with trained samples, the chimpanzee was informed whether her choice was correct or incorrect. On probe trials, which were inserted once every 10 trials on the average, the choice response was followed by neither the 'correct' nor 'error' feedback sound. Proportion correct is shown for the trained items as well as for the new colour and/or object introduced in each stage of training. The numbers in parentheses show the number of trials on which the proportions are based. The last column shows the proportion correct on the trials for the trained sample items, including all the colours and objects previously used. Generalization of the number name to new objects of familiar colour, to new colours of familiar objects, and to new objects in a new colour was at first poor. Less generalization was observed with new objects than new colours in each stage of training. As additional numbers were introduced and new objects and colours were used as exemplars, the correct number names were applied not only to new colours but also to new objects.

* $P<0.05$; † $P<0.01$; ‡ $P<0.001$ significantly above the chance level (χ^2 test).

most errors (94.9%) were confusions between neighbouring numbers.

Following acquisition of a new number name, generalization to a new object and/or a new colour was tested. The specific objects and colours used as well as test performance are summarized in Table 1 and reported in more detail elsewhere[13].

Accuracy during the final two sessions of naming 1, 2, 3, 4 and 5 with all five colours and five objects exceeded 98.5% in 830 trials. To reach this level of performance, a total of 95 sessions were required. In other words, 68 h 21 min (28,799 trials) elapsed from the beginning of training. It took an additional nine sessions (6 h 12 min, 3,004 trials) for Ai to reach criterion in selecting the correct Arabic numeral for groups of up to six red pencils.

How could Ai's newly acquired numerical skills be combined with object/colour naming? To answer this question, the chimpanzee was required to name not only the number of items, but also the colour and objects used to illustrate the number.

Fifteen keys, each of which represented one of five numbers (1, 2, 3, 4, 5), five colours (red, blue, yellow, green, black) and five objects (pencil, paper, brick, spoon, tooth-brush), were operative at one time. The sequence in which the keys were depressed was free, but the chimpanzee was required to select the three keys correctly describing each of the three attributes of $5\times5\times5=125$ types of sample items. For example, when five blue tooth-brushes were shown in the display window as a sample item, it was necessary for Ai to press keys of '5', 'blue' and 'tooth-brush' in any order. When the chimpanzee pressed an incorrect key, the 'error' sound followed and the trial ended. The pressed key was darkened and inoperative, so that the repeated response such as 'red/red' was inhibited.

Although no particular 'word order' was required, the chimpanzee favoured two particular sequences among the six possible alternatives, colour/object/number and object/colour/number (left panel of Fig. 3). In both sequences, numerical naming was always last. In the final sessions, it generally took less than 3 s for Ai to describe the object, colour and number of the sample items. She seldom looked back at the display between each component response. The response latency in the first component was the longest and the third component (numerical naming in almost all cases) was longer than the second one. The previously favoured sequences continued to be used for new samples with new colours and/or new objects (details in the right panel of Fig. 3).

These results suggest that the readiness with which the attributes are named is a partial determinant of the favoured sequences. Accuracy in numerical naming was always lower than that in object or colour naming in the three-term naming task. Moreover, number names were learned more slowly than were the names of objects and colours. It took 1,052 trials (171 min) for Ai to reach criterion in her first object discrimination task ('padlock' and 'glove'), 538 trials (105 min) for the first colour discrimination ('red' and 'green') and 1,821 trials (253 min) for the first number discrimination ('1' and '2'). In the previous stage of two-term naming, Ai had experienced both sequences, object/colour and colour/object. The previous experience may be the factor facilitating the reversible bonding of object and colour names in three-term naming. It seems that the favoured sequences in three-term naming are determined by complex factors, which include readiness with which the attributes are named and her previous experiences.

The range of numbers (1-6), the variety of sample items (at least 125 types), generalization to new items and the complex sequence of responses make it highly unlikely that the naming depends on the mere association of particular sample items and alternative keys. The present study suggests that the chimpanzee was able to match the sample items on the basis of numerosity in addition to colour and types of object.

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Co-localization of corticotropin releasing factor and vasopressin mRNA in neurones after adrenalectomy

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The discrete anatomical distribution of arginine vasopressin and corticotropin releasing factor (CRF) immunoreactivity in the paraventricular nucleus (PVN) of the rat hypothalamus is altered after adrenalectomy[1–3]. Not only is the immunostaining of both peptides enhanced, but vasopressin immunoreactivity, normally confined to the magnocellular subdivision, becomes clear in a large percentage of CRF neurones in the parvocellular subdivision[2]. These changes in immunoreactivity may reflect changes in post-translational events, peptide metabolism or genomic activity that lead indirectly or directly to the enhanced expression of vasopressin. Here we report that levels of transcripts homologous to vasopressin messenger RNA increase in the PVN after adrenalectomy, in parallel with increases in vasopressin immunoreactivity. In fact, after adrenalectomy, vasopressin mRNA can be detected in CRF-immunoreactive neurones. These results indicate that a considerable degree of plasticity is retained by the adult neuronal genome of the rat and that this plasticity may be modulated by the endocrine environment.

CRF and vasopressin act synergistically to release adrenocorticotropic hormone (ACTH) from the anterior pituitary[4]. Recently, immunohistochemical studies have demonstrated that CRF-containing fibres in the median eminence arise from cells in the parvocellular subdivision of the PVN[5], whereas the greatest density of vasopressin-containing neurones is found in the magnocellular subdivision of the PVN[6]. After adrenalectomy, the anatomical distribution of these peptides becomes less discrete; vasopressin and CRF can be found co-localized in >70% of parvocellular neurones[2]. It has been suggested that the removal of adrenal steroids accounts for these changes in neuropeptide expression[7]. Because steroids regulate peptide synthesis by modulating genomic activity[8], it seemed possible that adrenal hormones regulate neuronal synthesis of vasopressin by influencing some process preceding mRNA translation. Therefore, we examined the effects of adrenalectomy on vasopressin mRNA levels in the PVN by applying *in situ* hybridization techniques in conjunction with standard

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