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Perceptual learning without perception

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The brain is able to adapt rapidly and continually to the surrounding environment, becoming increasingly sensitive to important and frequently encountered stimuli[1–4]. It is often claimed that this adaptive learning is highly task-specific, that is, we become more sensitive to the critical signals in the tasks we attend to[5–15]. Here, we show a new type of perceptual learning, which occurs without attention, without awareness and without any task relevance. Subjects were repeatedly presented with a background motion signal so weak that its direction was not visible; the invisible motion was an irrelevant background to the central task that engaged the subject's attention. Despite being below the threshold of visibility and being irrelevant to the central task, the repetitive exposure improved performance specifically for the direction of the exposed motion when tested in a subsequent suprathreshold test. These results suggest that a frequently presented feature sensitizes the visual system merely owing to its frequency, not its relevance or salience.

We repeatedly presented a dynamic random dot (DRD) display[16] with a small number of temporarily, coherently moving (signal) dots and a much larger number of randomly moving (noise) dots in the background. During this display, subjects conducted an identification task of letters that were presented at the centre of the display[17] so that motion was an irrelevant feature. The ratio of signal to noise dots was a constant 5% during exposure. This signal strength was below the thresholds on tests of direction identification, direction discrimination and coherent motion detection tasks, which were conducted before, during and after the exposure. This suggests that attention and awareness had no access to the motion signal during the letter identification task. Nevertheless, after repeated exposure to this 'invisible', 5% level of coherent motion in the background, a significant performance improvement was obtained only for the motion directions within a small range around the exposed coherent direction when tested with a DRD display with 10% coherent motion. That is, this subsequent test revealed perceptual learning as a result of mere exposure to an 'invisible' motion direction.

The first experiment consisted of an exposure stage that was preceded and followed by control test stages (Fig. 1a). In each trial of

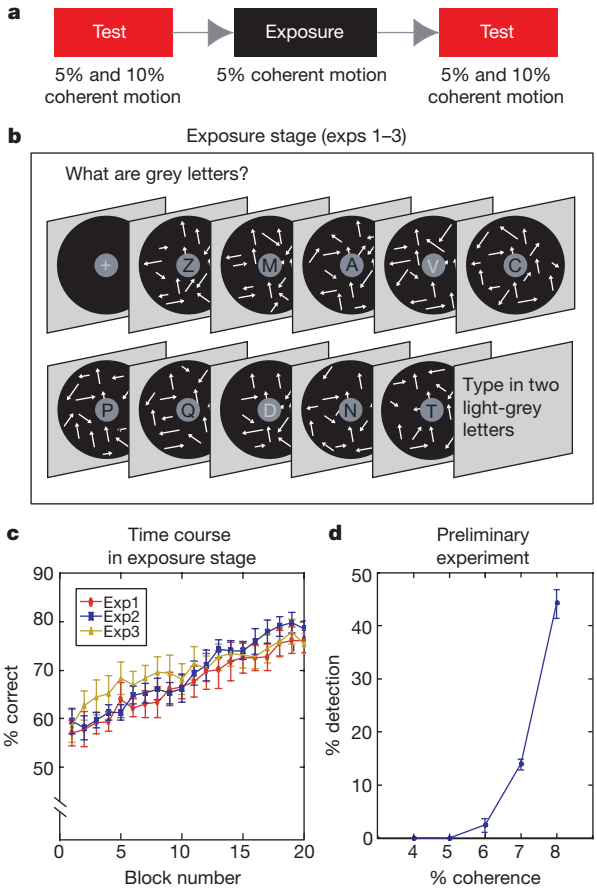

Figure 1 General procedure and exposure stage. **a**, In the exposure stage, dynamic random dot (DRD) displays with 5% coherent motion were presented; these were irrelevant to the task. In the test stages, DRD displays with 5% and 10% coherence were presented; these were relevant to the task. **b**, Exposure stage procedure. A sequence consisted of 8 black letters (0.5 cd m^{-2}) and 2 light-grey letters (53.0 cd m^{-2}) on a 1° diameter, dark-grey circle (32.0 cd m^{-2}), which was within the 10° diameter, black circular background (0.5 cd m^{-2}). Each letter was presented for 33 ms and was followed by a 17-ms blank interval. Thus, the duration of the sequence and the motion background was 500 ms. **c**, Time course of the mean 'correct' percentage (± s.e.) for eight subjects in the exposure stage in experiments 1–3. **d**, Mean percentage (± s.e. for eight subjects) of coherence detection as a function of coherence percentage in the preliminary experiment.

the exposure stage, ten letters were successively presented within a grey circle. During letter presentation, moving white dots were also presented on a surrounding black annulus background (Fig. 1b). Five per cent of the dots in a successive frame moved in the same direction while the remaining 95% of the dots were replaced, so that they were perceived to move randomly. This 5% coherence is lower than the detection threshold of 8.3% for coherent motion found in our preliminary experiment (see Methods and Fig. 1d). Direction of the coherent motion was determined randomly for each subject and was constant throughout the exposure stage. The subjects were instructed to identify two light-grey letters in order of their appearance in a rapidly changing stream of black letters presented at fixation, while ignoring the moving dots in the black background (Fig. 1b). To measure the effect of exposure on the performance for motion direction, the test stages were conducted before and after the exposure stage. In contrast to the exposure stage in which only 5% coherent motion was presented, in the test stages not only 5% but also 10% motion was presented to each subject. Whereas a coherent motion direction was constant for each subject in the exposure stage, in the test stages coherent motion direction was varied in eight steps from trial to trial. In each trial, the subjects were instructed to indicate which one of the eight coherent motion directions they perceived (Fig. 2a). Eye movements were monitored in all of the trials in the exposure and test stages.

The performance of letter identification in the exposure stage showed steady increase for all of the subjects (Fig. 1c). The performance obtained when tested with the 5% level of coherent motion before and after the exposure stage did not indicate any significant difference from chance level (Fig. 2b). This suggests that the subjects did not perceive precisely the 5% level of coherent motion during the exposure stage. When tested with 10% coherent motion in the test stages, performance improvement was significantly greater only for the exposed direction and its vicinity after the exposure stage, compared with before. These results were consistent across all of the subjects. Furthermore, there was no significant correlation between detected eye movements and the direction of coherent motion during the exposure stage, excluding the possibility that eye movements in the exposed coherent motion direction somehow improved performance in the second test stage.

To test whether the first test stage influenced performance in the

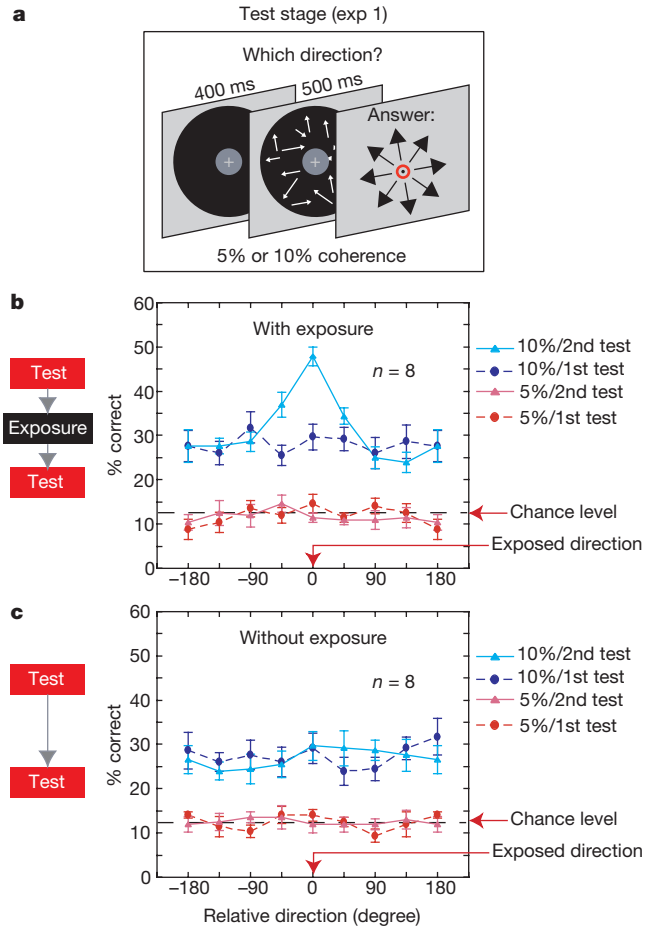

Figure 2 Test stages in experiment 1. **a**, Moving dots were replaced by eight arrows. Subjects placed a cursor on the arrow representing the perceived coherent direction. **b**, Mean correct percentage (± s.e.) for eight subjects. A level of 5% coherence produced no significant difference between correct percentage and chance, for any direction, either for the main and control conditions combined in the first test stage, or for the main or control condition in the second test stage (Wilcoxon signed rank (WSR) test). For 10% coherence, only for the exposed direction ($P < 0.01$, WSR test) and for –45° ($P < 0.05$, WSR test), the percentage was significantly higher after exposure than before. The mean correct percentage for all of the directions was significantly higher than chance ($P < 0.01$, WSR test). **c**, No significant difference was found in correct percentage between the two test stages in the control condition without the exposure stage (WSR test).

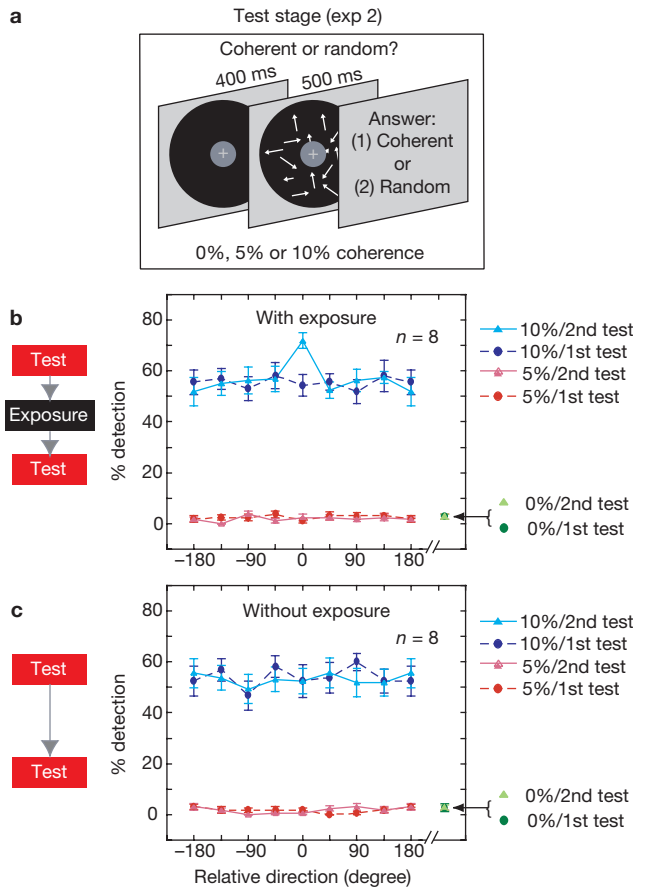

Figure 3 Test stages in experiment 2. **a**, After the disappearance of moving dots, subjects pressed a corresponding key to report whether coherent motion was perceived. **b**, Mean percentage (± s.e. for eight subjects) for the detection of 0%, 5% and 10% coherent motion. No significant difference was found between 5% and 0% coherent motion, for any direction, either for the main and control conditions combined in the first test stage or for the main or control condition in the second test stage (WSR test). For 10% coherence, the percentage was significantly higher after the exposure than before, only for the exposed direction ($P < 0.05$, WSR test). The percentage for all of the directions was significantly higher than for 0% coherent motion ($P < 0.01$, WSR test). **c**, No significant difference was found in detection percentage between the two test stages in the control condition without the exposure stage (WSR test).

second, eight new subjects were tested in the two test stages only, without the exposure stage. No improvement was found either for the 5% or 10% coherent motion condition (Fig. 2c).

The overall results of the first experiment indicate that performance on a task specifying a coherent motion direction improved through mere exposure to the motion direction, which was subthreshold and irrelevant to a task. To test whether the 5% coherent motion was actually invisible, we conducted a second experiment measuring the detectability of the coherent motion. The procedure for the exposure stage was identical to that used in the first experiment, and showed similar results (Fig. 1c). However, during the test stages in the second experiment, along with the 5% and 10% coherent motion displays, 0% (random motion) displays were also presented for each subject. The order of the presentation of these three kinds of displays was randomized from trial to trial. In each trial the subjects' task was to indicate whether they saw a group of dots moving coherently in the same direction (Fig. 3a).

The average likelihood of detecting the 5% coherent motion for any of the tested directions was nearly zero both before and after the exposure stage, and did not significantly differ from the average likelihood for the 0% condition (Fig. 3b). These results suggest that the 5% level of coherent motion was invisible (undetectable) during the exposure stage. When tested with the 10% level of coherent motion, detectability was significantly higher after exposure compared with before, only in the exposed motion direction. The results were consistent across all subjects. No effect of a preceding test stage on a subsequent test stage was found in the control without exposure stage (Fig. 3c).

We next tested whether the ratio of the number of 0% coherence motion trials to the total number of trials influenced the subjects' internal criterion responses. A control experiment was conducted with eight new subjects in which the number of trials for the 0% motion coherence condition was increased to 240, whereas the number of trials for each of 5% and 10% motion coherence was kept at the original 160. We obtained similar results (not shown) to those in the main condition (Fig. 2b, c), indicating that irrespective of the difference in the ratio of the 0% coherent motion trials to the total number of trials, coherent motion was not perceived when tested with the 5% level of coherent motion.

The results of the second experiment suggest that repeated exposure to a 5% coherent motion direction, although below detection threshold, resulted in enhanced detectability of the previously exposed motion direction when observed at a higher, more detectable 10% level of coherent motion.

In the first experiment, the difference between the closest motion directions represented by the arrows in the test stages was 45°. This raises the question of whether mere exposure to the 5% level of coherent motion enhances finer-scaled discriminability of the exposed coherent direction. Thus, in a third experiment, we tested discriminability of coherent motion directions that differed only by 3°. The procedure of the exposure stage was identical to that of the first experiment and showed similar results (Fig. 1c). In the test stages, the subjects were instructed to indicate whether the direction of coherently moving dots in two successively presented displays matched (Fig. 4a). In a 'matching trial' condition, the coherent motion directions in the two displays matched. In a 'non-matching trial' condition, the directions differed only by 3° (ref. 7). The same tendency as in the first experiment was observed (Fig. 4b). The lack of a significant difference between the subjects' performance and chance level in test stages before and after the exposure stage suggests that the 5% coherent motion was invisible (non-discriminable) during the exposure stage. The performance increase in discrimination of the previously exposed motion direction from those differing by only ± 3° when tested with 10% motion coherence suggests that mere exposure to the 5% invisible, coherent motion may fine tune the brain to the exposed motion direction. No effect of a preceding test stage on a subsequent test stage was found in the control without exposure stage (Fig. 4c).

In the above experiments, we found that the 5% level of coherent motion was neither discriminable nor detectable both before and after exposure to the motion. To further assure that subjects were unable to either detect or discriminate the 5% level of coherent motion while being exposed to it, in addition to the letter identification task the subjects performed a direction indication task (fourth experiment) or a coherent motion detection task (fifth experiment) in each trial of the exposure stage. Performance in the coherent motion indication task was near chance (12.5%) in all of the blocks of the exposure stages. The percentage of subjects detecting coherent motion was also not significantly different from 0% in any of the blocks of the exposure stage (not shown). These results further confirm that 5% coherent motion was indeed invisible throughout the exposure stage.

In all five experiments, with 5% coherent motion, performance in the direction indication, coherent motion detection and direction discrimination tasks in the test stages were all at chance level before, during and after the exposure phase. This provides compelling evidence that the 5% level of coherent motion was neither detectable nor discriminable, suggesting that attention and awareness had no

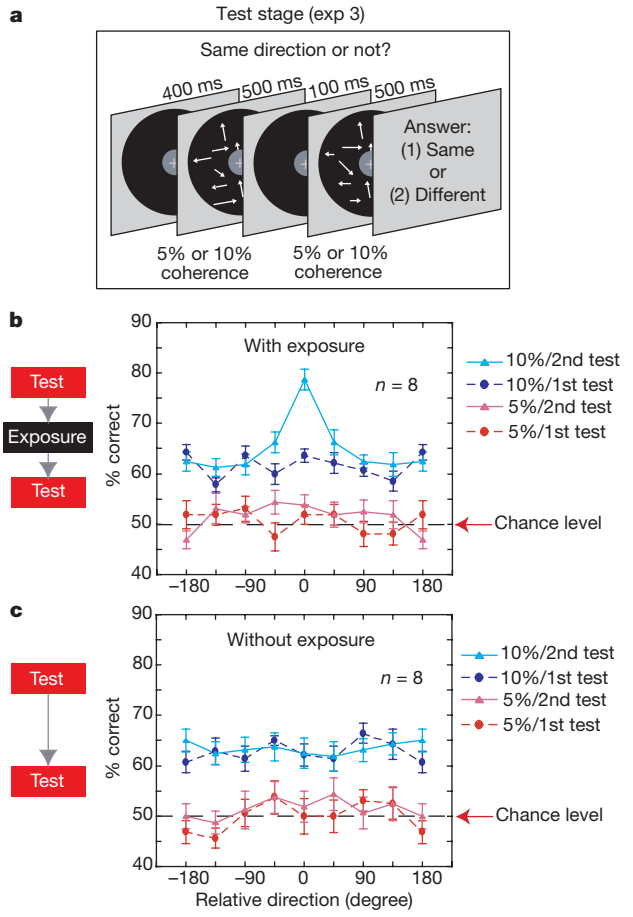

Figure 4 Test stages in experiment 3. **a**, Two DRD displays were presented, each for 500 ms with a 100-ms blank interval. After the disappearance of the second display, subjects pressed a corresponding key to report whether the coherent directions in the displays matched. **b**, Mean correct percentage (± s.e. for eight subjects) for 5% and 10% coherent motion, before and after the exposure. For 5% coherence, the same statistical results were obtained as in experiment 1. For 10% coherence, the percentage was significantly higher after the exposure than before for the exposed direction only ($P < 0.01$, WSR test). The percentage for all of the directions was significantly higher than chance ($P < 0.01$, WSR test). **c**, No significant difference was found in correct percentage between the two test stages in the control condition without the exposure stage (WSR test).

access to the motion signal during the letter identification task. Nevertheless, mere exposure to the 5% level of invisible, coherent motion resulted in enhanced performance specific for the exposed direction when subjects were subsequently tested with more visible (10%) coherent signals in all of the direction indication, coherent motion detection and direction discrimination tasks. We conclude that perceptual learning and modification of the underlying sensory mechanism specific for the exposed motion direction results from mere exposure to invisible, but physically present, motion.

Our findings have important implications for the question of how the brain adjusts its visual capabilities to the environment. It seems that frequent presentation of a feature, such as a particular direction of motion, sensitizes the visual system to that feature, even if the feature is perceptually invisible, and even if it is irrelevant to a given task. Although the present finding does not deny the important role of attention in perceptual learning[8,11,13–15] and in motion[18–22], it indicates that the adult brain has the flexibility to adapt to certain features of the environment as a result of mere exposure, even if the feature is so weak that it does not lead to awareness. This unsupervised modification would seem to have unwanted consequences: when we are bombarded by unimportant information we hope that by consciously ignoring it, we will escape its effects. This belief now seems unwarranted. The brain seems to equate frequency with ecological importance[23–28], and even though this may once have been a good strategy, it may be less adaptive in the context of our manipulative, modern-day media. It is possible that the present finding might be a result of unsupervised learning applied to lateral inhibition between neurons for different directions[29]. □

Methods

Subjects

The age of the subjects ranged between 19 and 25 years. All of the subjects had normal or corrected-to-normal vision and were naive to the purpose of the experiments. For each of the main and control conditions of all experiments, eight (experiments 1–3) or six (experiments 4–5) new subjects were used.

Preliminary threshold measurement

The 8.3% coherence motion detection threshold with the DRD display used in the test stages was established as a result of a preliminary experiment with the staircase method ($n = 6$). In the method, each time the subject's response (visible or invisible) changed, the direction of changes in signal-to-noise ratio (smaller or larger) is altered. The 10% coherent motion display was chosen to be used in the main experiments because its signal-to-noise ratio is over the established 8.3% threshold. It is uncertain whether subjects at no time perceived the coherent motion at the threshold. To determine the signal-to-noise ratio at which the subjects were unlikely to see the coherent motion, we asked eight new observers to report whether they saw coherent motion during 20 trials at each of 8, 7, 6, 5 and 4% coherence. None of the subjects reported any detection in the 5% and 4% coherence conditions (see Fig. 1d). Thus, we determined to use 5% coherence in the main experiments.

Design of the first experiment

The local dot speed was 14.2° per second in the motion displays. A non-optimal speed for detection of the coherent motion was chosen to ensure that detection would be poorer. The optimal speed, at least for a monkey, is known to be 3° per second[16]. Dot density was 1.27 dots per degree^2. Signal dots were randomly chosen in each frame. For example, for the 5% coherent motion display, 5% of the dots carried the signal from one frame to the next and then a different set of dots carried signals in the next frame transition[16]. Thus, the lifetime of almost all dots was the same as the frame duration of 50 ms.

For exposure stages, the first and second light-grey letters were presented in one of the first five serial positions and in one of the second five serial positions, respectively. They were determined randomly in each trial. Each block consisted of 960 trials and was completed on a different day. The exposure stage was terminated after 20 blocks (this was the number around which preliminary results indicated improvement asymptotes). No accuracy feedback was given to the subjects.

During the test stages each subject viewed the eight directions (−135°, −90°, −45°, 0°, 45°, 90°, 135° and 180°) from the exposed coherent direction, where positive and negative values represent clockwise and anticlockwise rotations. A trial for each direction was repeated 20 times. The order of the presentations of these conditions was randomly determined for each subject. No accuracy feedback was given to the subjects. The first test stage was conducted at least one day before the beginning of the exposure stage, and the second stage at least one day after the end of the exposure stage.

For measurements of eye movement, we used the ViewPoint Eye Tracker version 3 (Arrington Research) with approximately 0.3° resolution. For the control experiment with no exposure, subjects were given the same time interval between the two test stages (25 days), which was the mean interval in the main experiment (with exposure). The procedure of the test stages was identical to that of the main experiment.

Second experiment

Before the onset of the experiment, subjects were told that the number of trials in which coherent motion would be seen is not necessarily the same as that in the 0% coherent motion trials. Twenty trials were presented for each of the eight directions in the 5% and 10% coherent motion conditions, and 160 trials with the 0% coherent motion condition.

Third experiment

The test stages consisted of 50% matching trials and 50% non-matching trials. In the non-matching trials one of the eight directions (45° interval between the closest directions) was presented in the first display and the direction differing by ± 3° from that was presented in the second display.

Fourth and fifth experiments

In each trial of the exposure stage of the fourth experiment, both the letter identification task and the direction indication task, which was the same as in the test stages of the first experiment, were conducted. After the offset of a sequence of ten letters, three subjects were instructed first to conduct a direction identification task and then a letter identification task. The order of the tasks was reversed for the remaining three subjects. In the fifth experiment, instead of the direction indication task as in the fourth experiment, subjects performed the coherent motion detection task as in the test stage of the second experiment (Fig. 4a).

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Complete genome sequence of a multiple drug resistant *Salmonella enterica* serovar Typhi CT18

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***Salmonella enterica* serovar Typhi (*S. typhi*) is the aetiological agent of typhoid fever, a serious invasive bacterial disease of humans with an annual global burden of approximately 16 million cases, leading to 600,000 fatalities[1]. Many *S. enterica* serovars actively invade the mucosal surface of the intestine but are normally contained in healthy individuals by the local immune defence mechanisms. However, *S. typhi* has evolved the ability to spread to the deeper tissues of humans, including liver, spleen and bone marrow. Here we have sequenced the 4,809,037-base pair (bp) genome of a *S. typhi* (CT18) that is resistant to multiple drugs, revealing the presence of hundreds of insertions and deletions compared with the *Escherichia coli* genome, ranging in size from single genes to large islands. Notably, the genome sequence identifies over two hundred pseudogenes, several corresponding to genes that are known to contribute to virulence in *Salmonella typhimurium*. This genetic degradation may contribute to the human-restricted host range for *S. typhi*. CT18 harbours a 218,150-bp multiple-drug-resistance incH1 plasmid (pHCM1), and a 106,516-bp cryptic plasmid (pHCM2), which shows recent common ancestry with a virulence plasmid of *Yersinia pestis*.**

Salmonella typhi is a serovar of *S. enterica*, which is serologically O (lipopolysaccharide) type 09, 012; H (flagellin) type d; and Vi (extracellular capsule) positive. Humans are the only known natural host of *S. typhi*, with *S. typhi* showing limited pathogenicity for most animals. Isoenzyme analysis has suggested that isolates of *S. typhi* around the world are highly related[2], a view confirmed using multilocus sequence analysis (C. Kidgell *et al.*, unpublished data). Multiple drug resistance (MDR) is a serious emerging threat to the treatment of infectious diseases—MDR *S. typhi* are resistant to commonly available antibiotics, and clinical resistance to fluoroquinolones, the most effective antimicrobials for the treatment of typhoid fever, has been reported[3]. *Salmonella typhi* CT18 is an example of an emerging MDR microorganism; in depth genome analysis will contribute to our understanding of how such microorganisms adapt rapidly to new environmental opportunities that are presented by modern human society.

The principal features of the *S. typhi* CT18 chromosome and the two plasmids harboured by this strain are shown in Table 1. The beginning of the sequence was taken to correspond with minute 0 on the *E. coli* and *Salmonella* genetic maps[4]; the origin and terminus of replication, predicted by comparison with *E. coli* and confirmed by GC-bias (Fig. 1), are near 3.765 megabases (Mb) and 1.437 Mb, respectively. The metabolism of *Salmonella* and *E. coli* has been extensively studied over many decades[4], and our analysis reveals few surprises in this area. However, the chromosome was predicted to encode 204 pseudogenes, which is remarkable in a genome of an organism capable of growth both inside and outside the host. Most of these pseudogenes (124 out of 204) have been inactivated by the introduction of a single frameshift or stop codon, which suggests that they are of recent origin. Five pseudogenes (*priC*, *ushA*, *fepE*, *sopE2* and *fliB*) were re-sequenced from several independent *S. typhi* isolates, and were identical in every case. Frameshifts that are due to changes in the length of homopolymeric tracts account for 45 pseudogenes; this is a mechanism of variation that was previously shown to occur in *E. coli*[5], although at much lower rates than are required for rapid phase variation in other organisms. Some of the pseudogenes (27 out of 204) are the remnants of insertion sequence (IS) transposases, integrases and genes of bacteriophage origin. However, there are a significant number (75 out of 204) that are predicted to be involved in housekeeping functions, such as a component of the DNA primase complex, *priC*, cobalamin biosynthesis genes *cbiM*, *-J*, *-K* and *-C*, the proline transporter *proV*, and the anaerobic dimethyl sulphoxide reductase components *dmsA* and *dmsB*. Many more mutations (46 out of 204) are in genes that are potentially involved in virulence or host interaction. Examples of this latter group include components of seven of the twelve

Table 1 Features of the *S. typhi* genome

Component of genome	Property
Chromosome	
Total size	4,809,037 bp
G+C content	52.09%
Coding sequences	4,599
… of which pseudogenes	204
Coding density	87.6%
Average gene length	958 bp
Ribosomal RNAs	6 × (16S–23S–5S), 1 × (16S–23S–5S–5S)
Transfer RNAs	78
Other stable RNAs	8
pHCM1	
Total size	218,150 bp
G+C content	47.58%
Coding sequences	249
… of which pseudogenes	8
Coding density	83.8%
Average gene length	759 bp
pHCM2	
Total size	106,516 bp
G+C content	50.6%
Coding sequences	131
… of which pseudogenes	0
Coding density	87.1%
Average gene length	708 bp
Transfer RNAs	1