These data show that Dam⁻ *Salmonella* survive in Peyer's patches of the mouse small intestine for at least 5 days, providing an opportunity for elicitation of a host immune response. Dam⁻ *Salmonella*, however, were unable to cause disease; they either were unable to invade systemic tissues or were able to invade but could not survive.

DNA adenine methylases are potentially excellent targets for both vaccines and antimicrobials. They are highly conserved in many pathogenic bacteria that cause significant morbidity and mortality, such as *Vibrio cholerae* (*21*), *Salmonella typhi* (*22*), pathogenic *E. coli* (*23*), *Yersinia pestis* (*22*), *Haemophilus influenzae* (*24*), and *Treponema pallidum* (*25*). In addition, because Dam is a global regulator of genes expressed during infection (Fig. 1), Dam⁻ mutants may ectopically express multiple immunogens that are processed and presented to the immune system. Such ectopic expression could elicit a cross-protective immune response between related bacterial strains that share common epitopes. Finally, because the Dam methylase is essential for bacterial virulence, Dam inhibitors are likely to have broad antimicrobial action, hence Dam is a promising target for antimicrobial drug development.

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Sources of Mathematical Thinking: Behavioral and Brain-Imaging Evidence

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Does the human capacity for mathematical intuition depend on linguistic competence or on visuo-spatial representations? A series of behavioral and brain-imaging experiments provides evidence for both sources. Exact arithmetic is acquired in a language-specific format, transfers poorly to a different language or to novel facts, and recruits networks involved in word-association processes. In contrast, approximate arithmetic shows language independence, relies on a sense of numerical magnitudes, and recruits bilateral areas of the parietal lobes involved in visuo-spatial processing. Mathematical intuition may emerge from the interplay of these brain systems.

> Will it ever happen that mathematicians will know enough about the physiology of the brain, and neurophysiologists enough of mathematical discovery, for efficient cooperation to be possible? [Jacques Hadamard (*1*)]

Until recently, the only source of information about the mental representations used in mathematics was the introspection of mathematicians. Eloquent support for the view that mathematics relies on visuo-spatial rather than linguistic processes came from Albert Einstein, who stated: "Words and language, whether written or spoken, do not seem to play any part in my thought processes. The psychological entities that serve as building blocks for my thought are certain signs or images, more or less clear, that I can reproduce and recombine at will" (*2*). Many mathematicians report similar experiences (*1, 3*), but some have stressed the crucial role played by language and other formal symbol systems in mathematics (*4*). Still others have maintained that the critical processes giving rise to new mathematical insights are opaque to consciousness and differ from explicit thought processes (*1, 3, 5*).

We address the role of language and visuo-spatial representation in mathematical thinking using empirical methods in cognitive neuroscience. Within the domain of elementary arithmetic, current cognitive models postulate at least two representational formats for number: a language-based format is used to store tables of exact arithmetic knowledge, and a language-independent representation of number magnitude, akin to a mental "number line," is used for quantity manipulation and approximation (*6, 7*). In agreement with these models, we now demonstrate that exact calculation is language-dependent, whereas approximation relies on nonverbal visuo-spatial cerebral networks.

We first used behavioral experiments in bilinguals to examine the role of language-based representations in learning exact and approximate arithmetic. In one experiment, Russian-English bilinguals were taught a set of exact or approximate sums of two two-digit numbers in one of their two languages (*8*). In the exact addition condition, subjects selected the correct sum from two numerically close numbers. In the approximate addition condition, they were asked to estimate the result and select the closest number. After training, subjects' response times for solving trained problems and novel problems were tested in their two languages. Performance in both tasks improved considerably with training (response times dropped, in

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approximation, from 4423 to 2368 ms, and in exact calculation from 4285 to 2813 ms; both $P < 0.001$), regardless of the language in which a problem was trained (response times dropped from 4364 to 2644 ms in Russian and from 4344 to 2534 ms in English). Performance on exact and approximate tasks nevertheless showed different patterns of generalization during the test (Fig. 1). When tested on trained exact addition problems, subjects performed faster in the teaching language than in the untrained language, whether they were trained in Russian or English. This provided evidence that the arithmetic knowledge acquired during training with exact problems was stored in a language-specific format and showed a language-switching cost due to the required internal translation of the arithmetic problem. For approximate addition, in contrast, performance was equivalent in the two languages, providing evidence that the knowledge acquired by exposure to approximate problems was stored in a language-independent form.

Further evidence for contrasting representations underlying exact and approximate arithmetic came from comparisons of performance on trained problems and on novel problems involving similar magnitudes (Fig. 1). For exact addition, subjects performed faster on trained problems, suggesting that each new fact was stored independently of neighboring magnitudes, perhaps as a sequence of words. For approximate addition, performance generalized without cost to novel problems in the same range of magnitudes, providing evidence that new knowledge was stored using a number magnitude format (*9*).

A second experiment extended this phenomenon to more complex arithmetic tasks. A new group of bilinguals was taught two new sets of exact addition facts (two-digit addition with addend 54 or 63), two new exact operations (base 6 and base 8 addition), and two new sets of approximate facts (about cube roots and logarithms in base 2), with one task of each type trained in each of their languages (*10*). Over training, performance again showed large and comparable improvements for all tasks and for both languages. The exact tasks again exhibited large costs for language-switching and for generalization to novel problems for both languages of training, indicating language-specific learning, whereas the approximate tasks showed language- and item-independence (Fig. 1). These results suggest that the teaching of some advanced mathematical facts such as logarithms and cube roots can give rise to a language-independent conceptualization of their magnitude. Exact arithmetic, however, consistently relies on language-based representations (*11*).

To examine whether partly distinct cerebral circuits underlie the observed behavioral dissociation, two functional brain imaging techniques were used, one with high spatial resolution and one with high temporal resolution. Functional magnetic resonance images (fMRI) and event-related potentials (ERPs) were acquired while subjects performed tightly matched exact and approximate addition tasks (Fig. 2) (*12*).

In fMRI, the bilateral parietal lobes showed greater activation for approximation than for exact calculation. The active areas occupied the banks of the left and right intraparietal sulci, extending anteriorily to the depth of the postcentral sulcus and laterally into the inferior parietal lobule (Talaraich coordinates of main peaks: 44, −36, 52, $Z = 6.37$; 20, −60, 60, $Z = 6.03$; −56, −44, 52, $Z = 5.96$; −32, −68, 56, $Z = 5.10$) (Fig. 3). Activation was also found during approximation in the right precuneus (4, −60, 52, $Z = 4.99$), left and right precentral sulci (−56, 12, 24, $Z = 5.81$; 48, 16, 20, $Z = 4.80$), left dorsolateral prefrontal cortex (−44, 64, 12, $Z = 4.46$), left superior prefrontal gyrus (−32, 8, 64, $Z = 4.75$), left cerebellum (−48, −48, −28; $Z = 4.74$) and left and right thalami (12, −16, 16; $Z = 4.43$; −20, −8, 16, $Z = 4.04$).

Most of these areas fall outside of traditional perisylvian language areas (*13*), and are involved instead in various visuo-spatial and analogical mental transformations (*14–16*). Cortices in the vicinity of the intraparietal sulcus, in particular, are active during visually guided hand and eye movements (*15*), mental rotation (*16*), and attention orienting (*17*). Previous brain-imaging experiments also reported strong inferior parietal activation during calculation (*18*), although its functional significance could not be ascertained because of task-difficulty confounds. Here, the parietal activation cannot be attributed to eye movement, hand movement, and attentional or task difficulty artifacts because the approximate and exact tasks were matched in difficulty and in stimulus and response characteristics (*19*). Rather, it is compatible with the hypothesis that approximate

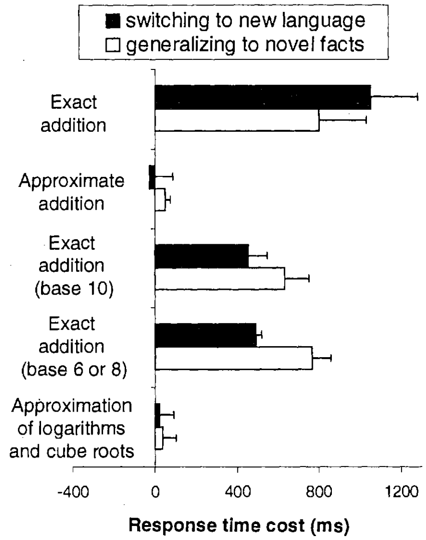

Fig. 1. Generalization of learning new exact or approximate number facts. Mean response times (RTs) to trained problems in the trained language are subtracted from RTs to trained problems in the untrained language (language cost: black bars) and from untrained problems in the trained language (generalization cost: gray bars). In experiment 1 (top two tasks), an analysis of variance on testing RTs indicated significant language-switching [$F(1,3) = 10.53$, $P < 0.05$] and generalization costs [$F(1,3) = 37.64$, $P < 0.01$] for the exact task, but no significant effect for the approximate task (both Fs < 1). The interactions of task (exact or approximate) on each cost measure were also significant [respectively, $F(1,6) = 11.10$, $P < 0.02$ and $F(1,6) = 24.71$, $P < 0.005$]. These effects were observed both with testing in English and with testing in Russian, and performance was similar in the two languages (for trained problems, mean RTs were 3445 ms in Russian and 3272 ms in English). In experiment 2 (bottom three tasks), similar analyses of variance indicated language-switching and generalization costs for base 10 addition, $F(1,7) = 24.23$, $P < 0.005$ and $F(1,7) = 28.61$, $P < 0.001$, and for addition in base 6 or 8, $F(1,7) = 304.06$, $P < 0.001$ and $F(1,7) = 71.10$, $P < 0.001$, but not for logarithm or cube root approximation (both Fs < 1). The interactions of task (exact or approximate) with each cost measure were also significant [respectively, $F(2,14) = 13.06$, $P < 0.001$ and $F(2,14) = 17.31$, $P < 0.001$]. Again, these effects were observed both with Russian and with English testing, and performance was similar in the two languages (for trained problems, mean RTs were 2639 ms in Russian and 2621 ms in English). Error rates were low in both experiments and were not indicative of speed-accuracy trade-offs.

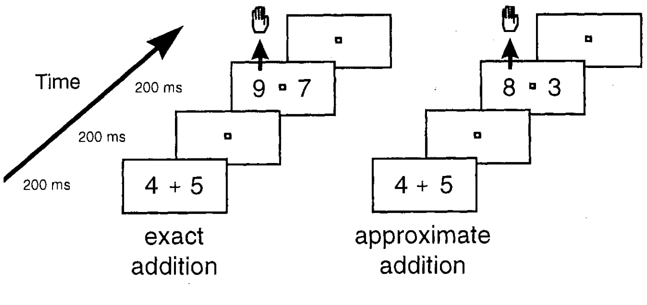

Fig. 2. Design of the tasks used during brain imaging. Subjects fixated continuously on a small central square. On each trial, an addition problem, then two candidate answers were flashed. Subjects selected either the correct answer (exact task) or the most plausible answer (approximate task) by depressing the corresponding hand-held button as quickly as possible. The same addition problems were used in both tasks (*12*).

calculation involves a representation of numerical quantities analogous to a spatial number line, which relies on visuo-spatial circuits of the dorsal parietal pathway.

The converse fMRI contrast of exact calculation relative to approximation revealed a large and strictly left-lateralized activation in the left inferior frontal lobe (–32, 64, 4, Z = 7.53) (*20*). Smaller activation was also found in the left cingulate gyrus (−8, 60, 16, Z = 6.14), left precuneus (−8, −56, 20, Z = 5.64), right parieto-occipital sulcus (20, −80, 28, Z = 5.27), left and right angular gyri (40, –76, 20, Z = 5.07; −44, –72, 36, Z = 4.99), and right middle temporal gyrus (48, –16, 8, Z = 4.68). Previous studies have found left inferior frontal activation during verbal association tasks, including generating a verb associated with a given noun (*21*). Together with the left angular gyrus and left anterior cingulate, these areas may constitute a network involved in the language-dependent coding of exact addition facts as verbal associations (*6*).

Because of their low temporal resolution, fMRI data are compatible with an alternative interpretation that does not appeal to dissociable representations underlying exact and approximate calculation. According to this alternative model, in both the exact and approximate tasks, subjects would compute the exact result using the same underlying representation of numbers. Differences in activation would be entirely due to a subsequent decision stage, during which subjects would select either an exact match or a proximity match to the addition result. The higher temporal resolution afforded by ERPs, however, shows that this alternative interpretation is not tenable. Crucially, ERP to exact and approximate trial blocks already differed significantly during the first 400 ms of a trial, when subjects were viewing strictly identical addition problems and had not yet received the choice stimuli (Fig. 3B). At 216 ms after the onset of the addition problem, ERPs first became more negative for exact rather than for approximate calculation over left inferior frontal electrodes, with a topography compatible with the fMRI activation seen in this same area. Previous ERP and intracranial recordings during the verb generation task also reported a latency of about 220 to 240 ms for the left inferior frontal activation (*22*). Later on in the epoch, starting at 272 ms after addition onset, ERPs became more negative for approximation over bilateral parietal electrodes, with a topography compatible with the bilateral parietal activation seen in fMRI. Thus, the recordings suggest that the two main components of the calculation circuits—the left inferior frontal activation for exact calculation and the bilateral intraparietal activation for approximation—are al-

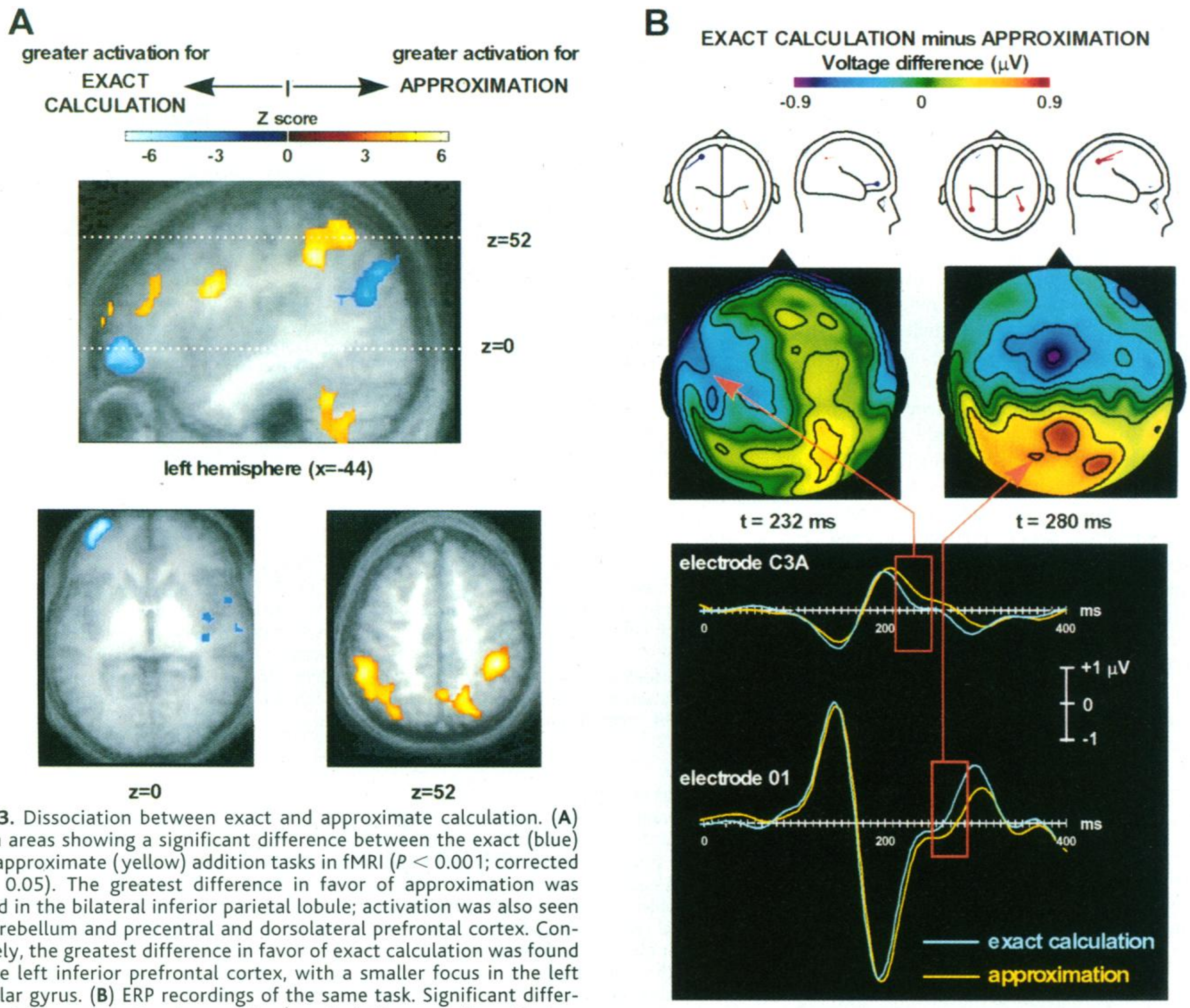

Fig. 3. Dissociation between exact and approximate calculation. (**A**) brain areas showing a significant difference between the exact (blue) and approximate (yellow) addition tasks in fMRI ($P < 0.001$; corrected $P < 0.05$). The greatest difference in favor of approximation was found in the bilateral inferior parietal lobule; activation was also seen in cerebellum and precentral and dorsolateral prefrontal cortex. Conversely, the greatest difference in favor of exact calculation was found in the left inferior prefrontal cortex, with a smaller focus in the left angular gyrus. (**B**) ERP recordings of the same task. Significant differences between exact and approximate calculation were found in two distinct time windows (red rectangles, $P < 0.05$), for which polar maps and dipole models of the corresponding interpolated voltage differences are shown. By 216 to 248 ms after the onset of the addition problem, ERPs were more negative during exact calculation over left inferior frontal sites (left). By 256 to 280 ms, ERPs were more negative during approximate calculation over bilateral parietal sites (right).

ready active at about 230 and 280 ms poststimulus. This demonstrates that the calculation itself, not just the decision, is performed using distinct circuits depending on whether an exact or an approximate result is required.

This conclusion is also strengthened by previous neuropsychological observations of patients with calculation deficits, in whom the lesion localization fits with the present fMRI results. Several lesion sites can cause acalculia (*23*). However, on closer examination, at least two distinct patterns of deficit are found (*24*). Some patients with left parietal lesions exhibit a loss of the sense of numerical quantity (including an inability to decide which number falls between 2 and 4 or whether 9 is closer to 10 or to 5), with a relative preservation of rote language-based arithmetic such as multiplication tables (*24, 25*). Conversely, aphasia following left-hemispheric brain damage can be associated with a selective impairment of rote arithmetic and a preserved sense of quantity, including proximity and larger-smaller relations between numbers (*24*). Particularly relevant to the present work is the case of a severely aphasic and alexic patient with a large left-hemispheric lesion who could not decide whether 2 + 2 was 3 or 4, indicating a deficit for exact addition, but consistently preferred 3 over 9, indicating preserved approximation (*26*). Thus, lesion data confirm that distinct circuits underlie the sense of quantity and knowledge of rote arithmetic facts.

In conclusion, our results provides grounds for reconciling the divergent introspection of mathematicians by showing that even within the small domain of elementary arithmetic, multiple mental representations are used for different tasks. Exact arithmetic puts emphasis on language-specific representations and relies on a left inferior frontal circuit also used for generating associations between words. Symbolic arithmetic is a cultural invention specific to humans, and its development depended on the progressive improvement of number notation systems (*27*). Many other domains of mathematics, such as the calculus, also may depend critically on the invention of an appropriate mathematical language (*28*).

Approximate arithmetic, in contrast, shows no dependence on language and relies primarily on a quantity representation implemented in visuo-spatial networks of the left and right parietal lobes. An interesting, though clearly speculative, possibility, is that this language-independent representation of numerical quantity is related to the preverbal numerical abilities that have been independently established in various animals species (*29*) and in human infants (*30*). Together, these results may indicate that the nonverbal representation that underlies the human sense of numerical quantities has a long evolutionary history, a distinct developmental trajectory, and a dedicated cerebral substrate (*31*). In educated humans, it could provide the foundation for an integration with language-based representations of numbers. Much of advanced mathematics may build on this integration.

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8. Participants were three female and five male bilingual Russian-English speakers, aged 18 to 32 years (mean age, 22.5 years), who began to learn English at a mean age of 15.3 years, had been in the United States for an average of 4.9 years and demonstrated fluent comprehension of both Russian and English on formal testing. All were undergraduate or graduate students, and all performed accurately on a variety of elementary arithmetic problems administered in Russian and in English during a pretest. Participants were trained on 12 sums of two two-digit numbers, totaling between 47 and 153. On each trial, an addition problem and two candidate answers were presented on a computer screen in word form, either in English or in Russian. Subjects selected one of the two answers, which appeared left and right of center, by pressing a corresponding key with the left or right hand. For exact addition, the candidate answers were the exact answer and a distractor in which the tens digit was off by one unit. For approximate addition, they were the multiple of ten closest to the correct sum, and another multiple off by 30 units. Each subject participated in 2 days of training with a fixed language and task (for example, exact addition in Russian) which was randomized across subjects (six repetitions of the 12 problems per day). Subjects also were trained for 2 days in a multiplication task (E. Spelke and S. Tsiukin, data not shown) with the same range of numbers in their other language, thus equalizing exposure to the two languages. On the fifth day, subjects were tested twice on each trained problem and twice on 12 similar untrained problems. Testing was done both in the original language of training and in the other language in different blocks.
9. The fact that subjects can estimate the solution of simple addition problems does not necessarily entail that the underlying mental representations are approximate. The present experiments only allow us to conclude that these representations are language-independent and encode numerical proximity.
10. Participants were four male and four female native Russian speakers, aged 18 to 24 years (mean age, 19.8 years), who were introduced to English at a mean age of 15.4 years, had lived in the United States for an average of 3.8 years, and fluently comprehended both Russian and English. All were undergraduate and graduate students who performed accurately in Russian and English pretests of elementary arithmetic skills. Each subject was trained in one language on 12 two-digit base-10 addition problems with addend of 54, 12 base-6 addition problems with two- to three-digit answers, and 12 cube-root estimation problems for numbers under 5000. The same subject was trained in the other language on 12 two-digit base-10 addition problems with addend of 63, 12 base-8 addition problems with two- to three-digit answers, and 12 base-2 logarithm estimation problems for numbers under 8500. Subjects received 2 days of training in each language, with six training trials per problem per training day. Order of training problems and pairings of problems and training languages were counterbalanced across subjects. Subjects received 1 day of testing in each of their languages on all the trained problems, plus an equal number of novel problems within the same six categories (two test trials per problem per testing day).
11. Anecdotal reports have suggested that when bilinguals calculate, they often revert to the original language in which they acquired arithmetic facts [P. A. Kolers, *Sci. Am.* **218**, 78 (March 1968); B. Shanon, *New Ideas Psychol.* **2**, 75 (1984)]. Furthermore, bilinguals solve arithmetic problems with greater speed and accuracy when the problems are presented in their first language [C. Frenck-Mestre and J. Vaid, *Mem. Cogn.* **21**, 809 (1993); L. G. Marsh and R. H. Maki, *ibid.* **4**, 216 (1976); L. McClain and J. Y. Shih Huang, *ibid.* **10**, 591 (1982)]. These observations, however, might simply reflect easier word comprehension and production processes in the first language, rather than a language-dependent encoding of arithmetic knowledge itself [M. McCloskey, P. Macaruso, T. Whetstone, in *The Nature and Origins of Mathematical Skills*, J. I. D. Campbell, Ed. (Elsevier, Amsterdam, 1992), pp. 493–537]. Our results, by contrast, showed a language-switching cost for exact calculation regardless of whether training was in the first or second language. Indeed, the cost of switching from the subjects' first language (Russian) to their second language was no greater than the cost of switching in the reverse direction (519 and 810 ms, respectively, for all exact tasks combined).
12. Participants were right-handed French students aged between 22 and 28 years (three men and four women in the fMRI version, five men and seven women in the ERP version). The project was approved by the regional ethical committee, and all subjects gave written informed consent. Stimuli were addition problems with addends ranging from 1 to 9 and sums ranging from 3 to 17. Ties such as 2 + 2 were excluded. For the exact task, the two candidate answers were the correct result and a result that was off by, at most, two units. In 90% of exact problems, the wrong result was of the same parity as the correct result, thus preventing the use of a short-cut based on parity checking [L. E. Krueger and E. W. Hallford, *Mem. Cogn.* **12**, 17 (1984)]. For the approximation task, the two alternatives were a number off by one unit, and a number off by at least four units. A third control task of letter matching was also introduced, in which digits were replaced by the corresponding uppercase letter in the alphabet, and subjects depressed the button on the side on which a letter was repeated from the initial pair. Tasks were presented in runs of alternating blocks of trials with a 4-s intertrial interval, separated by resting periods of 24 s. Four such runs were presented in semi-random order, two runs alternating exact calculation (three blocks of 18 trials each) with letter matching (three blocks of nine trials each), and two similar runs alternating approximation with letter matching. For fMRI, we used a gradient-echo echo-planar imaging sequence sensitive to brain oxygen-level dependent contrast (30 contiguous axial slices, 5 mm thickness, TR = 4 s, TE = 40 ms, angle = 90°, field of view 192 mm by 256 mm, matrix = 64 by 64) on a 3-T whole-body system (Bruker, Germany). High-resolution anatomical images (three-dimensional gradient-echo inversion-recovery sequence, TI = 700 ms, TR = 1600 ms, FOV = 192 mm by 256 mm, matrix = 256 × 128 × 256, slice thickness = 1 mm) were also acquired. Analysis was performed with SPM96 software (www.fil.ion.ucl.ac.uk/spm). Images were corrected for subject motion, normalized to Talairach coordinates using a linear transform calculated on the anatomical images, smoothed (full width at half maximum = 15 mm), and averaged across subjects to yield an "average run" in each condition (the results were replicated when the same analysis was applied to individual data with 5-mm smoothing). The generalized linear model was used to fit each voxel with a linear combination of two functions modeling early and late hemodynamic responses within each type of experimental block. Additional variables of noninterest modeled long-term signal variations with a high-pass filter set at 320 s. Because approximate and exact calculation blocks were acquired in different runs, the statistics we report used the interaction term (exact calculation – its letter control) – (approximate calculation – its letter control), with a voxelwise significance level of 0.001 corrected to

$P < 0.05$ for multiple comparisons. In a separate session, ERPs were sampled at 125 Hz with a 128-electrode geodesic sensor net reference to the vertex [D. Tucker, *Electroencephalogr. Clin. Neurophysiol.* **87**, 154 (1993)]. We rejected trials with incorrect responses, voltages exceeding $\pm$100 μV, transients exceeding $\pm$50 μV, electro-oculogram activity exceeding $\pm$70 μV, or response times outside a 200- to 2500-ms interval. The remaining trials were averaged in synchrony with stimulus onset, digitally transformed to an average reference, band-pass filtered (0.5 to 20 Hz), and corrected for baseline over a 200-ms window before stimulus onset. Experimental conditions were compared within the first 400 ms by sample-by-sample t tests, with a criterion of $P < 0.05$ for five consecutive samples on at least eight electrodes simultaneously. Two-dimensional maps of scalp voltage were constructed by spherical spline interpolation [F. Perrin, J. Pernier, D. Bertrand, J. F. Echallier, *Electroencephalogr. Clin. Neurophysiol.* **72**, 184 (1989)]. Dipole models were generated with BESA [M. Scherg and P. Berg, *BESA—Brain Electric Source Analysis Handbook* (Max-Planck Institute for Psychiatry, Munich, 1990)]. Three fixed dipoles were placed at locations suggested by fMRI (left inferior frontal and bilateral parietal), and the program selected the dipole orientation and strength to match the exact-approximate ERP difference on a 216- to 280-ms time window, during which significant differences were found.

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20. In individual analyses ($P < 10^{-3}$, corrected), the finding of greater intraparietal activation during approximation than during exact addition was replicated in five of seven subjects, whereas significantly greater left inferior frontal activation during exact addition was observed in four of seven subjects.
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Discovery of a Small Molecule Insulin Mimetic with Antidiabetic Activity in Mice

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Insulin elicits a spectrum of biological responses by binding to its cell surface receptor. In a screen for small molecules that activate the human insulin receptor tyrosine kinase, a nonpeptidyl fungal metabolite (L-783,281) was identified that acted as an insulin mimetic in several biochemical and cellular assays. The compound was selective for insulin receptor versus insulin-like growth factor I (IGFI) receptor and other receptor tyrosine kinases. Oral administration of L-783,281 to two mouse models of diabetes resulted in significant lowering in blood glucose levels. These results demonstrate the feasibility of discovering novel insulin receptor activators that may lead to new therapies for diabetes.

The actions of insulin are initiated by its binding to the insulin receptor (IR), a disulfide-bonded heterotetrameric membrane protein (*1–3*). Insulin binds to two asymmetric sites on the extracellular α subunits and causes conformational changes that lead to autophosphorylation of the membrane-spanning β subunits and activation of the receptor's intrinsic tyrosine kinase (*4*, *5*). Insulin receptors transphosphorylate several immediate substrates (on Tyr residues) including insulin receptor substrate (IRS) proteins (*6*). These events lead to the activation of downstream signaling molecules. The function of the receptor tyrosine kinase is essential for the biological effects of insulin (*1–6*).

The pathogenesis of type 2, non–insulin-dependent diabetes mellitus (NIDDM) is complex, involving progressive development of insulin resistance and a defect in insulin secretion, which leads to overt hyperglycemia. The molecular basis for insulin resistance in NIDDM remains poorly understood. However, several studies have shown modest ($\approx$30 to 40%) decreases in IR number with tissues or cells from NIDDM patients (*7*).

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