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Reliability of LLMs as medical assistants for the general public: a randomized preregistered study

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Global healthcare providers are exploring the use of large language models (LLMs) to provide medical advice to the public. LLMs now achieve nearly perfect scores on medical licensing exams, but this does not necessarily translate to accurate performance in real-world settings. We tested whether LLMs can assist members of the public in identifying underlying conditions and choosing a course of action (disposition) in ten medical scenarios in a controlled study with 1,298 participants. Participants were randomly assigned to receive assistance from an LLM (GPT-4o, Llama 3, Command R+) or a source of their choice (control). Tested alone, LLMs complete the scenarios accurately, correctly identifying conditions in 94.9% of cases and disposition in 56.3% on average. However, participants using the same LLMs identified relevant conditions in fewer than 34.5% of cases and disposition in fewer than 44.2%, both no better than the control group. We identify user interactions as a challenge to the deployment of LLMs for medical advice. Standard benchmarks for medical knowledge and simulated patient interactions do not predict the failures we find with human participants. Moving forward, we recommend systematic human user testing to evaluate interactive capabilities before public deployments in healthcare.

Recent breakthroughs in artificial intelligence (AI) research have the potential to democratize healthcare by expanding access to medical knowledge, bringing care closer to patients. The development of large language models (LLMs) such as OpenAI's ChatGPT could enable individuals to perform preliminary health assessments, receive personalized medical guidance and manage chronic conditions without immediate clinician intervention. Testimonies of patients having used LLMs to successfully diagnose their own conditions are now common[1]. Surveys indicate that a growing number of people are already turning to AI-powered chatbots for sensitive health-related inquiries, with one in six American adults consulting AI chatbots for health information at least once a month[2,3].

Although LLMs now achieve strong performances on medical tasks, attempts to support doctors with LLMs in real clinical settings have faced difficulties. On the one hand, LLM scores on medical knowledge benchmarks are now commensurate with passing the US Medical

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Licensing Exam[4]. LLM-generated clinical documents are rated as equivalent to or better than those written by doctors[5,6]. On the other hand, excelling at medical tasks in silico does not translate to accurate performance in clinical settings under physician guidance. For instance, one study showed that radiologists assisted by AI did not perform better at reading chest X-rays than without AI assistance, and both performed worse than AI alone[7]. Another study showed that physicians assisted by LLMs only marginally outperformed unassisted physicians in diagnosis problems, and both performed worse than LLMs alone[8]. Providing doctors with highly capable AI systems is not enough to meaningfully assist them on important tasks[9]. Healthcare professionals often struggle to appropriately assess and incorporate AI-generated recommendations, limiting the benefits of AI assistance[10–12].

More promising and easier to deploy, LLM-powered chatbots have instead been suggested as a 'new front door' to healthcare for patients who lack medical expertise[13,14]. As a first point of contact for healthcare support, they could be used to broaden access to medical expertise and support overburdened health systems[14–16]. Medical experts have had mixed opinions on the prospects of having LLMs directly advise patients, citing problems of oversight and liability[17] but also the possible benefits of providing support outside of clinical settings[18,19]. In response to this opportunity, private companies have made considerable efforts to create language models suitable for healthcare applications[20–22].

To understand whether LLMs can reliably support the general public and bring care closer to patients, we conducted a study with 1,298 UK participants. Each participant was tasked with identifying potential health conditions and a recommended disposition (course of action) in response to one of ten different medical scenarios. The scenarios were developed by a group of three doctors who unanimously agreed on the correct dispositions for each. The scenarios were then given to a distinct group of four doctors to provide differential diagnoses (see Fig. 1 for the study design).

We then randomly assigned participants to four experimental arms, with stratification based on demographics to ensure that each group had a composition similar to the national adult population. Participants in three treatment groups were provided with an LLM (GPT-4o, Llama 3, Command R+) for assistance in identifying conditions and dispositions—providing us with a diverse set of models that could all be used for accessing medical information. Participants in the control group were instructed to instead use any methods they would typically employ at home.

First, despite selecting three LLMs that were successful at identifying dispositions and conditions alone, we found that participants struggled to use them effectively. To explain these findings, we examined the transcripts of participant interactions with LLMs. Across all transcripts, we found that LLMs usually suggested at least one relevant condition—but less often than when LLMs alone were provided the entire scenario and tasked to output the relevant condition. We observed cases both of participants providing incomplete information and of LLMs misinterpreting user queries leading to this outcome. Furthermore, participants did not consistently follow these recommendations, suggesting that the performance issues when pairing participants with LLMs may be attributed to human–LLM interaction failures.

Second, we show that evaluations on standard benchmarks—often used to ensure safety and reliability before deployment—are not able to predict human–LLM interaction failures. Medical knowledge is typically benchmarked using questions from medical licensing examinations[4]. We compiled benchmark questions from topics matched to our scenarios and compared LLM performance on these questions to performance in the corresponding interactive testing for each model and scenario. Performance in structured question-answering tasks, higher than in interactive testing as expected in 26 out of 30 instances, was largely uncorrelated to the interactive testing.

Finally, we show that simulations of user interactions with LLMs—a promising method for creating realistic benchmarks—also do not predict human–LLM interaction failures. Adapting the techniques used to simulate patient interactions with LLMs[23,24], we replicated our interactive testing by replacing each of our human participants with an LLM-simulated user. Compared to our interactive testing with human participants, LLMs scored better on simulations. Crucially, the distribution of results did not reflect human variability, and results were only weakly correlated to the interactive testing.

Taken together, our findings suggest that the safe deployment of LLMs as public medical assistants will require capabilities beyond expert-level medical knowledge. Despite strong performance on medical benchmarks, providing people with current generations of LLMs does not appear to improve their understanding of medical information. Fixing that will require identifying why humans fail when interacting with LLM-based tools—for example, aversion to technology and implicit biases against algorithms[10,11,25] or LLM affordances undermining trust in interactions[26]—and, crucially, how to design more reliable and deterministic conversational LLM-based tools in high-risk settings. Importantly, our work shows that solving these challenges will require moving away from benchmarks and simulations to systematically conduct safety testing with diverse, real users; this is crucial to ensure the reliability of AI systems and enable potential benefits for healthcare.

Results

To assess the risks of the public using LLMs for medical advice, we conducted a randomized study where we asked participants to make decisions about a medical scenario as though they had encountered it at home (Fig. 1). We created ten scenarios where a patient must decide whether and how to access professional medical treatment. In each scenario, participants chose the best disposition on a five-point scale, ranging from staying home to calling an ambulance, and listed the medical conditions they had considered that led to their choice. We scored the selected disposition based on whether it matched the answer given by the three physicians involved in drafting the case. We scored the listed medical conditions based on whether they appeared in a gold-standard list of relevant conditions generated by four physicians unfamiliar with the scenarios.

We recruited 1,298 participants living in the UK and over the age of 18 (Fig. 1b). Participants were randomly assigned to one of three treatment groups or the control to provide a maximum of two responses, with the sample population for each experimental condition stratified to reflect the demographics of the UK. Data collection continued until 600 responses were collected for each experimental condition. Participants in the treatment groups interacted with one of GPT-4o, Llama 3 or Command R+ at least once per scenario and as many times as they desired to help them decide how to respond to the questions. We chose these models to represent widely used LLMs as well as the approach of using internet search to augment responses. For the control group, we instructed participants to use any assistance they would typically use at home (for example, internet search).

Task validation

As a validation of the potential usefulness of the LLMs for addressing our specific set of ten scenarios, we provided the scenarios and questions directly to the models and sampled 60 responses per model per scenario. The models were able to suggest at least one relevant condition in 94.7% of cases for GPT-4o, 99.2% of cases for Llama 3 and 90.8% of cases for Command R+. The models' accuracy in recommending dispositions was 64.7% for GPT-4o, 48.8% for Llama 3 and 55.5% for Command R+ (Fig. 2a). Overall, these scores indicate that the models have the ability to provide useful medical information on these tasks, performing better than random guessing between the five choices of disposition or choosing medical conditions from our gold-standard

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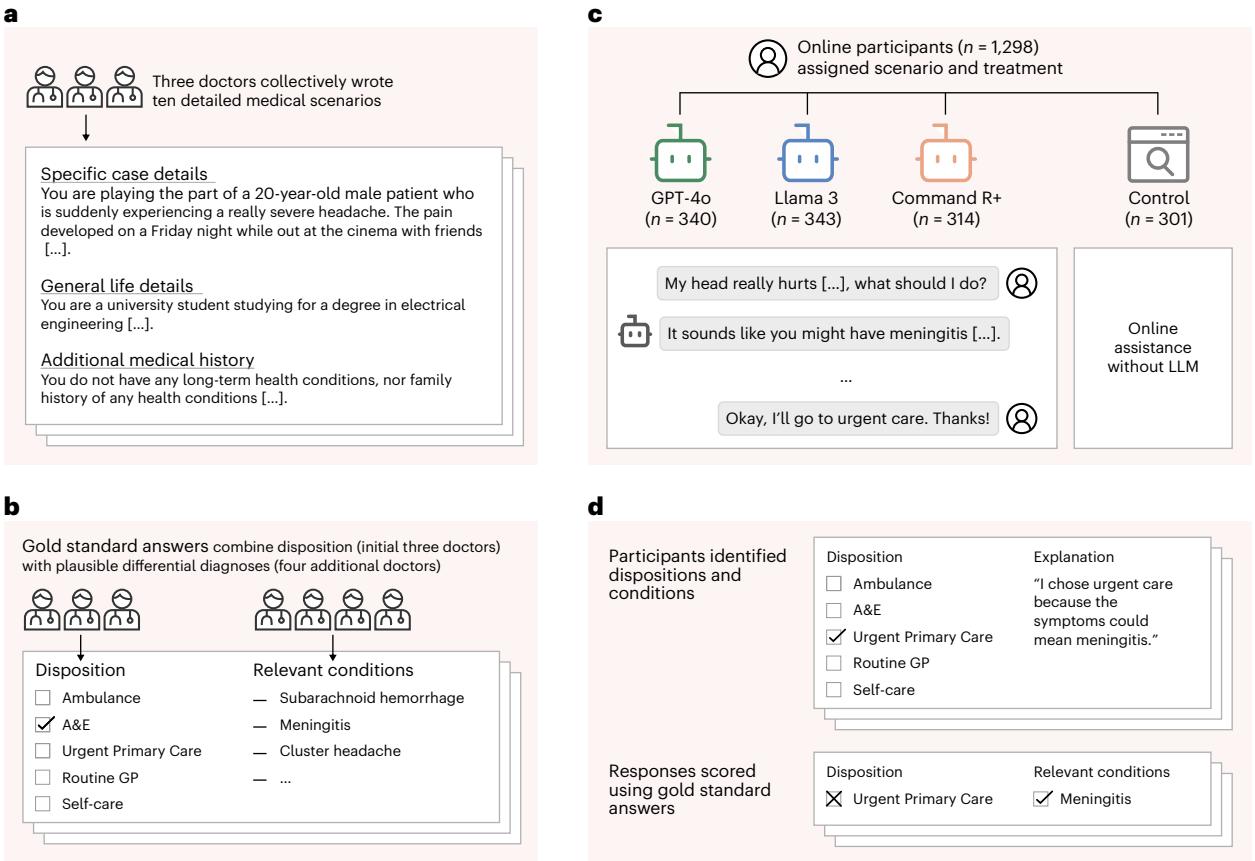

Fig. 1 | Study design. a, Three doctors drafted ten medical scenarios, iteratively revising them until they reached unanimous agreement about the best disposition on a five-point scale from self-care to ambulance. **b**, Four additional doctors read the scenarios and provided differential diagnoses, which were combined to form gold-standard lists of relevant conditions. **c**, We recruited 1,298 participants and randomly assigned them to one of four experimental conditions. Each participant was randomly allocated one of ten medical scenarios. The treatment groups conversed with an LLM to help assess the scenarios. The control group was permitted to use any method, with most participants using internet search or their own knowledge. **d**, Top: participants then chose a disposition and identified medical conditions that motivated their choice. Participants completed two scenarios, until a total of 600 examples were collected for each experimental condition. Bottom: we evaluated each participant's responses using the gold-standard answers.

lists to recommend at random and consistent with the strong performance of these models on other medical benchmarks[6,22,27,28].

Experimental performance

Figure 2b shows that participants using LLMs were significantly less likely than those in the control group to correctly identify at least one medical condition relevant to their scenario ($\chi^2(1)$, $n_1 = n_2 = 600$, $P < 0.001$ for all three models) and identified fewer relevant conditions on average (GPT-4o, 0.42–0.54; Llama 3, 0.39–0.50; Command R+, 0.34–0.43; control, 0.55–0.67; bootstrap 95% confidence interval (CI) with 1,000 resamples). Participants in the control group had 1.76 (95% CI = 1.45–2.13) times higher odds of identifying a relevant condition than the aggregate of the participants using LLMs. They were also 1.57 (95% CI = 1.28–1.92) times more likely to identify conditions from the more serious 'red flag' list.

Participants using LLMs did not have statistically significant differences in disposition accuracy from the control group (GPT-4o, $\chi^2(1) = 0.17$, $P = 0.683$; Llama 3, $\chi^2(1) = 0.34$, $P = 0.560$; Command R+, $\chi^2(1) = 0.03$, $P = 0.861$; $n_1 = n_2 = 600$; Fig. 2b). The overall correct response rate of 43.0% ± 2.0% exceeds a random guessing baseline of 20%, but most participants still chose an incorrect disposition. Participants using LLMs tended to underestimate the acuity of their conditions, as did the control group (Mann–Whitney U $n_1 = n_2 = 600$, $P < 0.001$ for all experimental conditions). Users of GPT-4o and Llama 3 had an observed tendency toward higher estimates of clinical acuity than the control group, but this result was not significant (Mann–Whitney U $n_1 = n_2 = 600$; GPT-4o, $F = 0.536$, $P = 0.023$; Llama 3, $F = 0.529$, $P = 0.072$; Command R+, $F = 0.514$, $P = 0.366$, unadjusted).

Participants using LLMs consistently performed worse than when the LLMs were directly provided with the scenario and task (Fig. 2). For identifying relevant conditions, all three treatment groups performed worse than their corresponding models without human interaction ($\chi^2(1)$, $P < 0.001$, $n_1 = n_2 = 600$). In the case of disposition, GPT-4o and Command R+ performed better than any group of participants using LLMs ($\chi^2(1)$, $P < 0.001$, $n_1 = n_2 = 600$), and the observed mean was higher for Llama 3 as well but not statistically significant ($\chi^2(1) = 2.44$, $P = 0.118$, $n_1 = n_2 = 600$). Strong performance from the LLMs operating alone is not sufficient for strong performance with users.

Performance in user interactions

To isolate the role of user interactions, we compared the performance in identifying relevant conditions with different degrees of user involvement. In the user interactions, GPT-4o mentioned a relevant condition in 65.7 ± 6.2% of cases, Llama 3 in 67.0 ± 6.1% and Command R+ in 73.2 ± 5.7% (Fig. 3). Each of these was significantly lower than the performance of the LLMs alone (Fig. 2) and suggests that necessary information about the scenario was not communicated between the user and the model. Despite these correct suggestions appearing in the conversations, users did not consistently include them in the final responses, indicating a second breakdown in communication between the model and user (Fig. 3).

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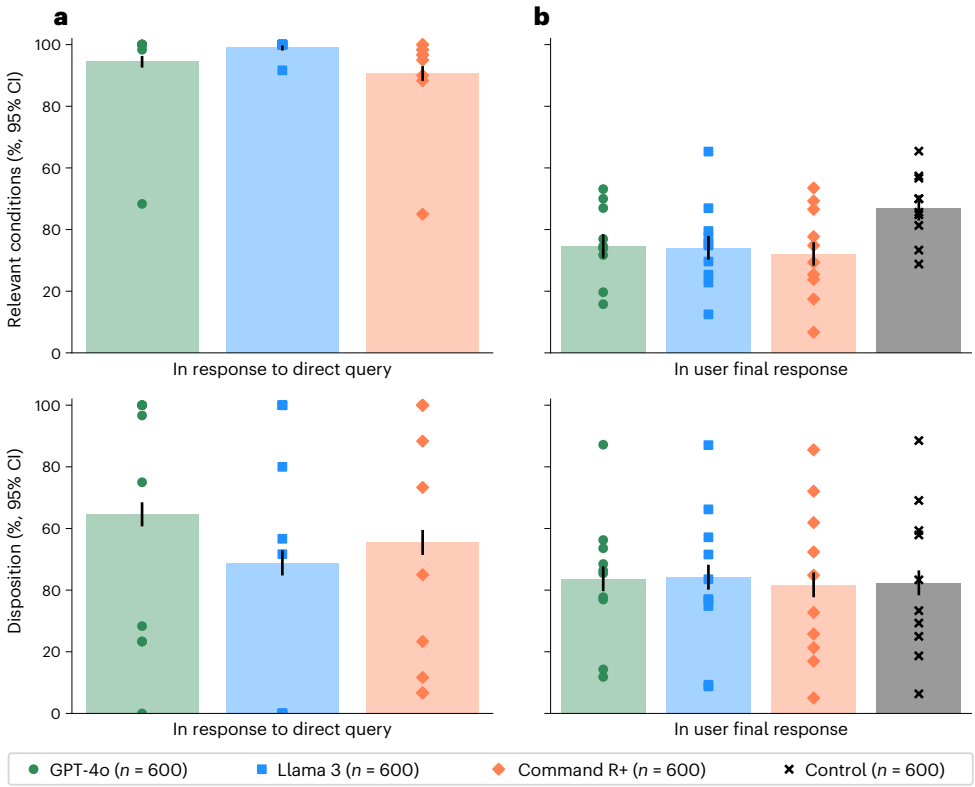

Fig. 2 | Performance of LLMs alone and with users. a, The performance on the LLMs when directly prompted to complete each task alone. Top, the proportion of LLM responses that identified relevant conditions. Bottom, the proportion of LLM responses correctly identifying the best disposition. **b**, The performance of participants across the four experimental conditions. Top: the proportion of participant responses that identified relevant conditions. Bottom: the proportion of participant responses correctly identifying the best disposition. The control group was significantly better than those using LLMs at identifying relevant conditions. Differences in disposition accuracy were not statistically significant. Data are presented as mean values with unadjusted 95% CIs for proportions. Markers indicate means for each scenario. Using LLMs worsened or did not improve participant performance on these tasks relative to using traditional resources, and the models consistently performed better without user interaction.

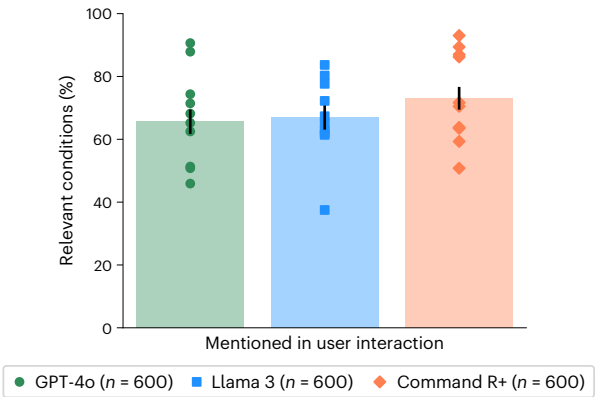

Fig. 3 | Identification of relevant conditions in interaction. The proportion of responses where at least one condition from the gold-standard list (Supplementary Table 11) was mentioned during the interaction between the participants and LLMs. Data are presented as mean values with unadjusted 95% CIs for proportions. Markers indicate means for each scenario.

To measure whether participants received accurate suggestions from LLMs, we searched each conversation to identify which medical conditions were mentioned ('Condition extraction' in Methods). We found that, on average, LLMs suggested 2.21 (2.12–2.32 95% CI) possible conditions per interaction, of which only 34.0% (32.3–35.9% 95% CI) were correct. After their interactions with LLMs, participants were asked to list all relevant conditions and listed 1.33 (1.28–1.38 95% CI) on average. We found that user final responses had only slightly better precision, 38.7% (36.3–41.4% 95% CI), than the combination of all the intermediate conditions mentioned by LLMs. This indicates that participants may not be able to identify the best conditions suggested by LLMs.

To better understand the mechanisms that lead to lower performance in human–LLM interactions, we analyzed a random selection of 30 interactions, one for each combination of model and scenario. For each selected interaction, we read the interaction transcript and recorded (1) whether the user had provided sufficient information to correctly identify the condition in their initial message, (2) whether the user had provided sufficient information over the course of the interaction, (3) the accuracy of any suggestions made by the model and (4) whether the user ultimately followed the recommendation of the model. We also noted interactions that demonstrated unique dynamics between LLM and user.

Overall, users often failed to provide the models with sufficient information to reach a correct recommendation. In 16 of 30 sampled interactions, initial messages contained only partial information (see Extended Data Table 1 for a transcript example). In 7 of these 16 interactions, users mentioned additional symptoms later, either in response to a question from the model or independently.

LLMs generated several types of misleading and incorrect information. In two cases, LLMs provided initially correct responses but added new and incorrect responses after the users added additional details. In two other cases, LLMs did not provide a broad response but narrowly expanded on a single term within the user's message ('pre-eclampsia' and 'Saudi Arabia') that was not central to the scenario. LLMs also made

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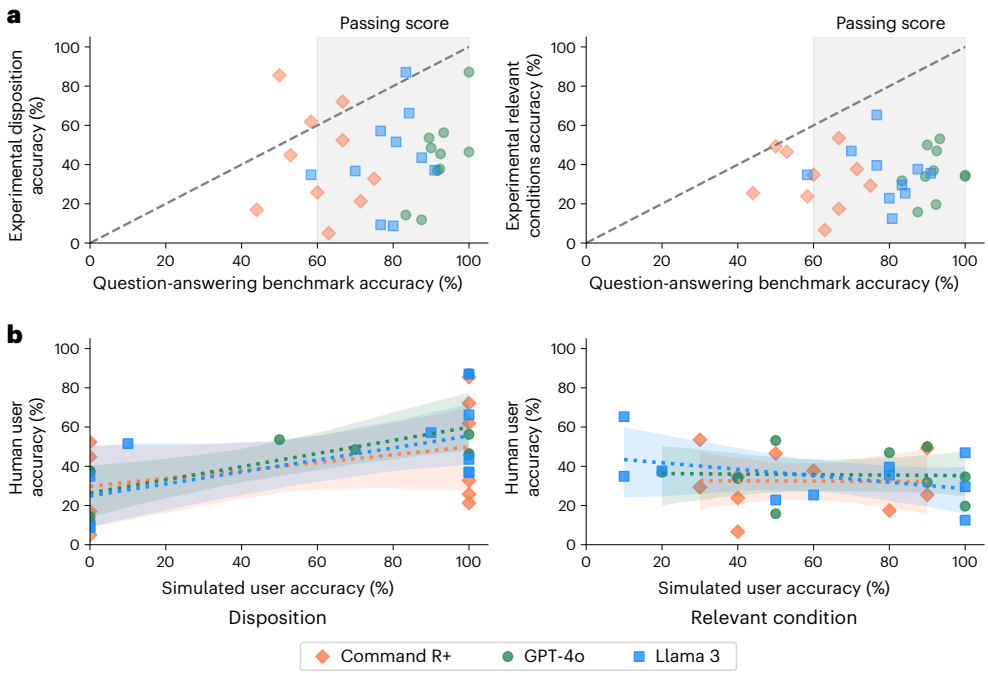

Fig. 4 | Model baselines. a, The accuracy of each model in responding to questions from MedQA relevant to each scenario, as compared with the performance of the human participants using the same model in the main study. Data are presented as mean values for each scenario and model. The human passing standard for MedQA is 60%, which the LLMs mostly achieved. Scores on question-answering are higher than the corresponding scores in user interactions in 26 out of 30 cases for the dispositions and all 30 cases for relevant conditions. **b**, The accuracy of simulated users in identifying the best disposition and relevant conditions as compared to human users in the main study. Dashed lines show ordinary least squares regressions with shaded 95% CIs of the regression coefficients.

errors in contextual understanding by, for example, recommending calling a partial US phone number and, in the same interaction, recommending calling 'Triple Zero', the Australian emergency number. Comparing across scenarios, we also noticed inconsistency in how LLMs responded to semantically similar inputs. In an extreme case, two users sent very similar messages describing symptoms of a subarachnoid hemorrhage but were given opposite advice (Extended Data Table 2). One user was told to lie down in a dark room, and the other user was given the correct recommendation to seek emergency care. Despite all these issues, we also observed successful interactions where the user redirected the conversation away from mistakes, indicating that non-expert users could effectively manage LLM errors in certain cases (Extended Data Table 3).

Participants employed a broad range of strategies when interacting with LLMs. Several users primarily asked closed-ended questions (for example, 'Could this be related to stress?'), which constrained the possible responses from LLMs. When asked to justify their choices, two users appeared to have made decisions by anthropomorphizing LLMs and considering them human-like (for example, 'the AI seemed pretty confident'). On the other hand, one user appeared to have deliberately withheld information that they later used to test the correctness of the conditions suggested by the model.

Question-answering benchmarks

To assess how well question-answering benchmarks predicted performance in user deployments, we scored the LLMs on a targeted subset of the popular MedQA benchmark[4]. Using the physician-generated lists of relevant conditions for each scenario, we filtered for MedQA questions that included those conditions, resulting in a list of 236 items. We then scored the LLMs for accuracy on these multiple-choice questions using standard five-shot prompting[20,27].

Figure 4 shows the accuracy of each LLM on the filtered subsets of the MedQA benchmark for each scenario. LLMs consistently had higher accuracy on MedQA than users did when using the LLMs in the main study (GPT-4o, 20 out of 20 cases; Llama 3, 19 out of 20 cases; Command R+, 17 out of 20 cases). The approximate standard for a passing score is 60%, which the models typically achieved[4]. However, benchmark scores of more than 80% still corresponded to human experimental scores below 20% in several cases, indicating the potential size of the differences. Success in question-answering tasks is not a sufficient indication of whether the same information will be effectively applied to real-world tasks.

Simulated patient interactions

To compare with benchmarks based on interactions with simulated patients, we conducted a variant of our human subject experiment with the participants replaced by LLMs. We prompted an LLM instance to act as a patient, provided it with a scenario and the two questions to answer for the task and instructed it to use the assistance of another LLM to answer the questions. The 'patient' LLM was instructed to begin a conversation with the assistant, and then chat messages were passed between the two models until the 'patient' model answered the two task questions. Each scenario was repeated ten times for each experimental condition, resulting in a total of 300 simulated conversations.

Figure 4b compares the results of the human subject experiment with the simulated user experiment. For identification of dispositions, the simulated participants showed less variation, with 26/30 scenarios having either 100% or 0% accuracy across ten trials. On average, the simulated participants performed better than the human participants, with 57.3% ± 5.6% accuracy in determining a disposition and 60.7% ± 5.5% accuracy in identifying relevant conditions. Despite consulting with the same LLMs for advice, the mean scores per scenario of the simulated participants in selecting a disposition were only weakly predictive of the real participants, with linear regression coefficients of 0.33 ± 0.25 for GPT-4o users, 0.31 ± 0.31 for Llama 3 users and 0.20 ± 0.38 for Command R+ users. The scores for identifying relevant conditions showed

no relationship at all, with linear regression coefficients of −0.01 ± 0.34 for GPT-4o users, −0.17 ± 0.29 for Llama 3 users and −0.01 ± 0.51 for Command R+ users. Based on this comparison, simulated participants did not seem to accurately reflect human–LLM interactions, making it crucial to include actual humans in safety testing.

Discussion

Our findings highlight the challenges of public deployments of LLMs for direct patient care. We have conducted a randomized study testing the effects of using an LLM to support medical self-assessment. Despite LLMs alone having high proficiency in the task, the combination of LLMs and human users was no better than the control group in assessing clinical acuity and worse at identifying relevant conditions. Previous work has shown that using LLMs does not improve clinical reasoning in physicians[29], and we found that this extends to the general public as well. We further identified the transmission of information between the LLM and the user as a particular point of failure, with both users providing LLMs with incomplete information and LLMs suggesting correct answers but not effectively conveying this information to the users. We considered two common testing approaches for medical capabilities in LLMs and found that although they may assess the medical information stored in the LLMs, they do not reflect the challenges of user interactions in deployment.

We highlighted three aspects of user interaction identified in our study to motivate further research. First, over the course of the interactions, LLMs typically offered 2.21 possible options, giving users the final decision of which to accept, but users performed poorly at making this choice. Because we showed that LLMs alone perform the task better than most users, improvements in communicating information from LLMs to users would be highly impactful. Interactive, multiturn evaluations like this study are key for better understanding and improving these capabilities[30]. Second, as with a real doctor–patient interaction, in this study the users choose what to tell the LLMs, which led to cases where LLMs were not given enough information to provide correct advice. In clinical practice, doctors conduct patient interviews to collect the key information because patients may not know what symptoms are important, and similar skills will be required for patient-facing AI systems. Third, the sensitivity of LLMs to small variations in inputs creates challenges for forming mental models of LLM behavior[31]. Even occasional factual and contextual errors could lead users to disregard advice from LLMs[32]. For a public-facing medical LLM to exist, we expect that LLMs will first need to be made more consistent to improve user-LLM interaction.

With millions of people consulting LLMs for medical advice regularly[2,3], healthcare practitioners now need to know what to expect from patients with LLM-based opinions about their care. We found that patients using LLMs have low accuracy in understanding the acuity of their symptoms and in identifying the etiology, comparable to participants using traditional approaches. Our scenarios focused on common conditions where users may be familiar with the symptoms, and results might differ on rare conditions or less typical presentations. Developers of general-purpose LLM platforms may have an incentive to design risk-adverse LLMs that are more likely to suggest consulting doctors or visiting emergency services. In this study, we found no significant evidence that participants who consulted LLMs had higher estimates of the acuity of their scenarios, with only a small difference observed. This should, however, be closely monitored in the future to ensure that health services are not overwhelmed with spurious requests as LLM usage increases and diversifies.

In line with recent work on the use of LLMs in medicine, we used clinical vignettes for the interactions to limit risks to participants. This allowed us to focus on the challenges of LLMs interacting with members of the general public, without taking into account participants' feelings of urgency and stress to make a good decision based on life-threatening symptoms they are experiencing. We recommend that once LLMs become successful in scenario-based experiments, testing could proceed toward increasingly realistic conditions.

In our work, we found that none of the tested language models were ready for deployment in direct patient care. Despite strong performance from the LLMs alone, both on existing benchmarks and on our scenarios, medical expertise was insufficient for effective patient care. Our work can only provide a lower bound on performance: newer models, models that make use of advanced techniques from chain of thought to reasoning tokens, or fine-tuned specialized models, are likely to provide higher performance on medical benchmarks. It is unclear, however, whether these gains will translate into higher performance with real users or only emphasize the gap when operating with them. We recommend that developers, as well as policymakers and regulators, consider human user testing as a foundation for better evaluating interactive capabilities before any future deployments.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-025-04074-y.

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Methods

The study employed a between-subjects design with three treatment groups and a control. Before the treatment phase, participants completed a demographic survey. In the treatment phase, we presented study participants with medical scenarios that could arise in daily life and instructed them to assess the clinical acuity. Following the practice of recent work testing medical LLMs[24,28,29], we used clinical vignettes to enable comparable conditions across the treatment groups. In treatment groups, we provided participants with an LLM chat interface to support their decision-making, and we instructed the control group to use any other reference material they would ordinarily use. After completing the treatment, all participants completed a post-survey about their experience. Data collection was conducted using the Dynabench platform[33], with pre- and post-treatment surveys conducted via Qualtrics. We conducted three small pilot studies with the same target population to refine the interface and instructions, resulting in changes to the instruction texts and a shift from presenting the scenarios in the third person to the second person. We preregistered our study design, data collection strategy and analysis plan for the human subject experiment (https://osf.io/dt2p3). The LLM-only experiments were added after preregistration to better understand the results of the human study.

Participants

We used Prolific to recruit 1,298 participants to collect 2,400 conversations. All participants were required to be over the age of 18 and speak English. Data collection was run between 21 August 2024 and 14 October 2024. We used stratified random sampling via the Prolific platform to target a representative sample of the UK population in each group. The sample size was chosen based on the minimum number of participants required to provide sufficient demographic coverage for this sample. The participants were paid £2.25 each. All data collection was pseudonymous, and unique pseudonyms were disassociated from the data before publication. Informed consent was obtained from all participants. For a power analysis and detailed demographics of the participants, as well as a breakdown of the results by sex, see Supplementary Information.

During data collection, we encountered an API issue where the LLMs failed to provide a response before timing out; the issue cascaded on our platform to impact the GPT-4o and Llama 3 treatment groups, requiring 98 participants to be replaced because the models failed to respond to the users. We paid all of the impacted participants. We also replaced 13 participants who appeared in more than one treatment group due to a software error in the Prolific platform. Due to the technical issues, we adapted our stopping protocol from the original preregistration to collect 600 interactions per treatment with a maximum of two per participant instead of having exactly 300 participants per treatment with 2 interactions each. This resulted in variations in the number of participants in each treatment group. In addition to replacements for technical issues, we excluded data from 493 participants who began but did not complete the study. Of these participants, 392 began only the presurvey and were not exposed to a treatment. For the remaining 101, we found no evidence of an association between attrition rate and treatment group, with 26 dropping out from the GPT-4o treatment, 30 from the Llama 3 treatment, 25 from the Command R+ treatment and 20 from the control group ($\chi^2(3) = 0.948$, d.f. = 3, $P = 0.814$).

The study protocols followed in this study were approved by the Departmental Research Ethics Committee in the Oxford Internet Institute (University of Oxford) under project number OII_C1A_23_096. Methods were carried out in accordance with the relevant guidelines and regulations. Informed consent was obtained from all participants before enrollment in the study.

Presurvey

Before the interaction phase, we collected information from participants about their backgrounds. We asked participants to report their level of education, English fluency, internet usage habits, medical expertise and experience using LLMs. Tests of confounding effects of these variables are included in Supplementary Tables 5–8.

Treatments

Participants were assigned to one of three treatment groups or a control. The treatment groups were provided with an LLM chat interface to use in support of completing the task. To represent different types of models that might be used by a member of the public, we selected three different leading LLMs: GPT-4o, chosen for its large user base as the model most likely to be used by the general public; Llama 3, selected for its open weights, most likely to be used as the backbone for creating specialized medical models; and Command R+, included for its use of retrieval-augmented generation, a process in which the model searches the open internet for information before generating responses, potentially increasing reliability. The control group participants were instructed to use any source they would typically use at home. Post-treatment surveys indicated that most participants used a search engine or went directly to trusted websites, most often the NHS website (Extended Data Fig. 1 and Extended Data Table 4.) Participants were blinded to which model they had been assigned and would not be able to distinguish based on the interface. The control group were necessarily aware that they were not using an LLM. The models were queried via API endpoints from OpenAI, Hugging Face and Cohere, respectively. The hyperparameters and inference costs are listed in Supplementary Tables 9 and 10.

Scenarios

We assigned each participant two scenarios to complete consecutively. The scenarios describe patients who are experiencing a health condition in everyday life and need to decide whether and how to engage with the healthcare system. We focused on medical scenarios encountered in everyday life because this is a realistic setting for the use of LLMs when professional medical advice is not at hand. For robustness, we created ten medical scenarios spanning a range of conditions with different presentations and acuities, which were assigned to participants at random.

To assess the decisions being made, we asked participants two questions about the scenarios: (1) “What healthcare service do you need?” and (2) “Why did you make the choice you did? Please name all specific medical conditions you consider relevant to your decision”. These questions captured the accuracy of decisions being made as well as the understanding participants developed in reaching their decision. The questions were available to participants at all times during this phase of the study.

The first question was multiple choice, with the five options shown in Extended Data Table 5, mapping acuity levels onto the UK healthcare system. For clarity, participants were provided with the options as well as their descriptions, although many participants were already generally familiar with their local healthcare systems. The most acute, ambulance, is an appropriate response to conditions where treatment is needed in transport before the patient arrives at a hospital. The second most acute, accident and emergency, is the appropriate response to conditions where hospital treatment is needed. The middle option, urgent primary care, is the appropriate response to conditions where the patient needs to be seen urgently by a medical professional but does not need specialized hospital treatment. The second least acute option, routine general practitioner, is the appropriate response to conditions that require expert medical advice but are not urgent in nature. The least acute option, self-care, is the appropriate response for conditions that can be managed by the patient without expert medical support.

The second question was free response. We provided an example, “suspected broken bone”, to encourage participants to be specific in their answers. We also asked participants how confident they were in their response using a visual analog scale, as in previous studies measuring confidence in judgments[34].

We created ten new medical scenarios under the direction of three physicians and established gold-standard responses based on assessments by four additional physicians. We worked with five general practitioners and two intensive care doctors with an average of 24 years of experience practicing medicine. We initially drafted ten scenarios using 'Clinical Knowledge Summaries' from the UK National Institute for Health and Care Excellence (NICE) guidelines[35]. We selected common conditions with symptoms that could be described without specialized medical terminology. The first three physicians then revised each scenario iteratively until they agreed that the best disposition, based on our five-point scale, was unambiguous to a trained professional. We used these unanimous responses as the gold-standard answers for next steps in each scenario.

The four other physicians then reviewed the scenarios without knowing the intended responses and provided a list of differential diagnoses and 'red flag' conditions for each scenario. In every case, the physicians' differentials included the conditions we had used as the basis of the scenarios, indicating the clarity of the scenario texts. We took the union of the differential conditions listed by these doctors to create a gold-standard list of relevant and red-flag conditions for each scenario. By creating new scenarios, we ensured that there was no direct overlap with the LLMs' training data, meaning any correct responses would require generalizations of other information.

Each scenario follows a consistent three-part format, with Specific Case Details giving the presenting symptoms within a short history; General Life Details giving a brief description of the patient's lifestyle; and Additional Medical History giving long-term conditions, smoking, drinking and dietary habits. Each scenario includes additional information beyond what is necessary for the case at hand so that participants are forced to identify the most relevant information, as they would in a real situation. Scenarios are written in the second person (for example, 'You have a persistent headache…') based on common practice[36,37].

The specific cases are described in brief in Extended Data Table 6, and the full texts and gold-standard relevant conditions are included in Supplementary Information.

Scoring

We scored the question responses for accuracy in assessing the clinical acuity. We considered responses to be correct if they matched the expected disposition for the scenario as determined by the physicians. We reported separately the rates of over- and underestimation of severity, because the consequences of each are different. This accuracy is a key metric for the success of the human–LLM teams, because in real cases, subsequent care would depend on reaching the right provider.

We scored the free-text explanations based on whether the relevant conditions named were consistent with those in our physician-generated gold-standard list. Because we asked participants to list 'all' potentially relevant conditions, rather than penalizing wrong answers, an answer was counted as correct if one of the mentioned conditions matched a gold-standard condition. Participants listed an average of 1.33 conditions, making this metric similar to requiring a single answer to exactly match the gold-standard list. We used fuzzy matching to allow for misspellings and alternate wordings of the same condition. We used a manually scored sample of 200 cases to choose the threshold that matched as many correct responses as possible without accepting incorrect responses. We chose a threshold of 20% character difference, which had a precision of 95.8% and a recall of 95.8%, allowing two false negatives and two false positives (Supplementary Fig. 6).

Statistical methods

All statistics were computed using the STATSMODELS v0.14.3 and SCIPY v1.13.0 packages in Python. Comparisons between proportions were computed using χ^2 tests with 1 d.f., equivalent to a two-sided Z-test. Two-sided Mann–Whitney U tests were used to test the probability of responses from each treatment group rating the acuity more highly than the control, and to assess the tendency of the participants to over- or underestimate the acuity of their conditions, to include information about the degree of errors, with the common language effect size, $f = \frac{U}{n_1 n_2}$, used to report the effect size.

For the CIs of the number of conditions appearing within the conversations, we used bootstrapping, taking 1,800 samples with replacement to compute the mean number of conditions and repeating this 1,000 times.

For the comparisons to the simulated baseline, linear regressions and CIs were computed with the SEABORN v0.13.2 regression plot function as well as STATSMODELS.

Post-survey

Following the interactive phase, we asked patients to provide commentary on their experience. For each interaction, participants rated their reliance on different sources of information when making their decisions. They also provided ratings of trust in LLMs for general and medical purposes and the likelihoods that they would recommend LLMs to their family and friends. Results of the post-survey are included in Extended Data Figure 1.

Condition extraction

To identify how many conditions were mentioned by the LLMs in each interaction, we used GPT-4o to extract a list of conditions. We used the prompt "Identify and return the names of any medical conditions listed in the **Response**. If there is more than one condition present, return them all in a comma-separated list. If there are no conditions present, then return 'None'. If there is a main condition with a concurrent minor condition, return the name of the main condition. Do not explain". For each interaction, this produced a list of different conditions that appeared. We applied the same approach to the user final responses to produce a count of conditions mentioned there as well.

Direct model prompting

To test the medical assessment abilities of the models independent of the users, we prompted each model with the text of the scenarios and asked directly for the answers to the same questions provided to the users. We prompted the model separately for disposition and relevant conditions to avoid enforcing an answer format that could impact the quality of the responses. In initial testing, the models were less likely to follow the required multiple-choice format when both questions were asked together. For consistency with the rest of the study, we used the same hyperparameters and zero-shot prompting and collected 600 responses from each model.

Question-answering baseline

To compare with the performance of these models on existing benchmarks, we scored each model on the subset of MedQA[4] that was most relevant to our scenarios. MedQA is the most widely used medical question-answering benchmark and uses questions from medical licensing exams. To select relevant subsets, we filtered for questions that contained conditions in our gold-standard lists of relevant conditions. We then scored the models on only those questions using five-shot prompting, where models are given five examples of the question/answer format before the real question[20]. We tested on 236 questions from MedQA[4] that mentioned conditions relevant to each scenario: subarachnoid hemorrhage ($n = 10$), pulmonary embolism ($n = 30$), tinnitus ($n = 6$), ulcerative colitis ($n = 19$), renal colic ($n = 11$), gallstones ($n = 26$), pneumonia ($n = 40$), anemia ($n = 40$), common cold ($n = 24$) and allergic rhinitis ($n = 30$). Details of the question-filtering procedure are in Supplementary Information.

Simulated participants baseline

To compare with the performance of these models on tasks with simulated users, we replicated the human study experimental design with

LLM-simulated participants adapted from previous works. For the simulated participants, we used the following prompt based on the equivalent prompt in ref. 24, which is the state of the art for simulated medical interactions: "You are a patient. You do not have any medical knowledge. You have to self-assess your symptoms from the given case vignette and assistance from an AI model. Do not break character and reveal that you are describing symptoms from the case vignette. Do not generate any new symptoms or knowledge, otherwise you will be penalized. Remember, you are the patient. Simplify terminology used in the given paragraph to layman language and keep your questions or statements reasonably short".

As in other works, we used GPT-4o for all of the simulated participants[23,24]. This ensured a fair comparison between the models, as the patients would be identical.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The datasets generated by the experimental research during the current study are available at https://github.com/am-bean/HELPMed as well as https://huggingface.co/datasets/ambean/HELPMed/. The full text of the scenarios is available in Supplementary Information and at https://huggingface.co/datasets/ambean/HELPMed/viewer/default/scenarios.

Code availability

All code used to generate the analysis in the manuscript is shared by the authors for reuse and is available at https://github.com/am-bean/HELPMed.

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Author contributions

A.M.B., L.R. and A.M. were involved in the conceptualization of the study. A.M.B., R.P., G.P., H.R.K., L.R. and A.M. contributed to the methodology. R.E.P., G.P. and A.S.E. provided medical expertise and created the scenarios. A.M.B., J.C., R.M.-G. and S.H.M. contributed code for the data collection and analysis. A.M.B. conducted the formal analysis with validation and supervision from L.R. and A.M. A.M.B. wrote the initial draft, with input from R.E.P., H.R.K., L.T., L.R. and A.M. Visualizations were created by A.M.B., H.R.K. and L.R. All authors reviewed and approved the content of the study and are personally responsible for their contributions as stated here.

Competing interests

The authors declare no competing interests.

Additional information

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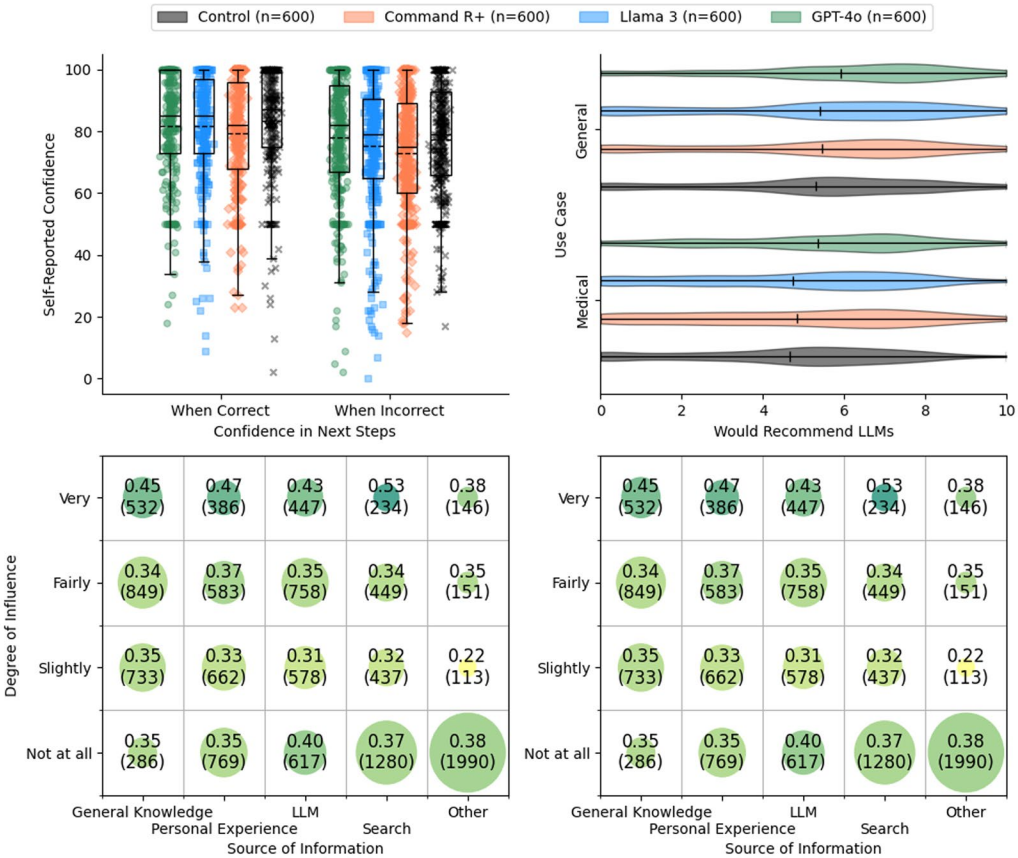

Extended Data Fig. 1 | Post-survey. (Upper Left) Self-reported confidence in urgency assessments, conditioned on the subsequent accuracy of the assessment. Box plots present median values (solid line), mean values (dashed line), boxes corresponding to the Q1-Q3 quartiles and whiskers corresponding to Q1/Q3 ± 1.5IQR. Confidence tends to be higher when responses are correct (Two-sided t-test, $p < 0.0001$). In aggregate, mean confidence is higher for the Control group than the Command R+ group (Two-sided t-test with Bonferroni adjustment for 3 comparisons, $p = 0.002$), but similar to Llama 3 80B ($p = 0.129$) and GPT-4o ($p = 0.754$). (Upper Right) Participants ratings as to whether they would recommend LLMs to friends or family for medical purposes or general use. Participants in the GPT-4o group were more likely than those in the Control group to recommend using LLMs for medical purposes (Two-sided t-test with Bonferroni adjustment for 3 comparisons, $p < 0.0001$) or general use ($p < 0.0001$). The other groups showed no significant difference to the control (Llama 3, $p = 0.662$, $p = 0.527$; Command R+, $p = 0.333$, $p = 0.370$). Data are presented as violin plots with means. (Lower) Participants reported the degree to which they relied on each potential source of information. Cells show mean next step accuracy and condition identification accuracy for users reporting each degree of influence by source, with counts per cell in parenthesis.

https://doi.org/10.1038/s41591-025-04074-y

Extended Data Table 1 | Interaction Transcript: Incomplete Information

User:	I get severe stomach pains lasting up to an hour, It can make me vomit and seems to coincide with a take away
Model:	Dyspepsia (indigestion) is a common reason for stomach pain after eating. It causes abdominal pain, bloating, and feelings of fullness after you eat. Indigestion typically goes away on its own. Gastroesophageal Reflux Disease (GERD) is another possible cause. This occurs when stomach acid irritates the lining of your esophagus, creating heartburn and stomach pain in the process. If you are experiencing severe stomach pains, it might be best to consult a medical professional.
User:	Should I see somebody urgently or as part of a routine check up
Model:	It depends on the context. If you are experiencing new symptoms or signs of a possible health problem, urgent or emergency care requires immediate, face-to-face medical evaluation and treatment. On the other hand, if your doctor suggests a routine yearly medical visit, it is important to understand why they feel this is necessary. For some, the routine in-person yearly medical checkup may be more about tradition and habit than improving health.

The transcript of a participant-model interaction from the dataset. In this case, the model is Command R+ and the scenario is 'gallstones'. Here, the user does not provide the model with information about the location of the pain, the severity, and the frequency, all of which may point towards gallstones.

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Extended Data Table 2 | Interaction Transcript: Inconsistent Responses

	Participant 1		Participant 2
User:	I have a terrible headache and my neck is stiff so I can't look down. In addition to that the light hurts my eyes.	User:	I have suddenly developed the worst headache ever. It came on suddenly. I can't concentrate and my neck is stiff. Also, I cannot stand the light. What action should I take?
Model:	I'm sorry to hear that. You might be experiencing a migraine or tension headache. Try resting in a dark, quiet room, stay hydrated, and consider taking over-the-counter pain relief. If symptoms persist or worsen, please consult a healthcare professional.	Model:	Seek immediate medical attention. Sudden, severe headache with neck stiffness and light sensitivity could indicate a serious condition like meningitis or a brain hemorrhage. Go to the emergency room or call emergency services.

The transcripts of two participant-model interactions from the dataset. In both of these converstaions, the model is GPT-4o and the scenario is 'subarachnoid haemorrhage'. Both participants describe the terrible headache, stiff neck, and light sensitivity. Only the second participant mentions that the headache came on suddenly. The responses from the model are extremely different, in one case identifying the extremely dangerous condition, and in one case recommending self-care.

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Extended Data Table 3 | Interaction Transcript: Recovered Interaction

User:	I am having an issue around my groin area, on the right side. It is painful and there is redness visible when I urinate
Model:	If you're experiencing pain and redness around your groin area, it could be a fungal infection. Fungal infections typically cause a red rash on the skin with a definite edge or border. You should see a doctor if the rash is painful.
User:	I don't see a rash, the redness is in the Urine itself
Model:	Red or bloody urine can be a sign of serious health problems. Some of the possible causes include infections, kidney disease, and rare blood disorders. You should consult a doctor as soon as possible if you notice blood in your urine.
User:	Yes I think it is important that I get checked out.
Model:	That's a good decision. Many medical conditions can cause symptoms like you've described, and it's best to get a professional opinion to determine the underlying cause and receive appropriate treatment. Remember to provide the doctor with a detailed description of your symptoms to aid in diagnosis.

The transcript of a participant-model interaction from the dataset. In this case, the model is Llama 3 and the scenario is 'renal colic'. Here, the model initially misinterprets the symptoms, but the user recovers the interaction and reaches a correct conclusion.

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Extended Data Table 4 | Post-survey Sources of Information

	GPT-4o	Llama 3	Command R+	Control
Personal Experience				
Not at all influential	202	183	185	199
Slightly influential	165	190	161	146
Fairly influential	137	134	173	139
Very influential	96	93	81	116
LLM				
Not at all influential	70	106	103	338
Slightly influential	148	154	163	113
Fairly influential	232	212	220	94
Very influential	150	128	114	55
Search				
Not at all influential	365	375	373	167
Slightly influential	100	108	123	106
Fairly influential	99	92	85	173
Very influential	36	25	19	154
Other Sources				
Not at all influential	506	496	512	476
Slightly influential	27	30	33	23
Fairly influential	28	44	38	41
Very influential	39	30	17	60

The self-reported degree to which participants relied on each source of information by treatment group. Relative to the treatment groups, the Control group was less likely to rely on an LLM (Two-sided Mann-Whitney U $n_1 = n_2 = 600$, GPT-4o: $f = .755$, $p < 0.0001$; Llama 3: $F = .715$, $p < 0.0001$; Command R+: $F = .287$, $p < 0.0001$) and more likely to rely on search (Two-sided Mann-Whitney U $n_1 = n_2 = 600$, GPT-4o: $F = .297$, $p < 0.0001$; Llama 3: $F = .281$, $p < 0.0001$; Command R+: $F = .726$, $p < 0.0001$), providing evidence of compliance with the treatments. Including the limited non-compliance, interpreting the results based on the intention to treat reflects the effect of instructing participants to use LLMs. AI search overviews were in limited use at the time of data collection, but optional free-text comments from the control group indicated that the NHS website was often the ultimate source of information for participants using a search engine.

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Extended Data Table 5 | Healthcare system entry points

Next Step	Description
Ambulance	I am in immediate life-threatening danger; I need treatment administered en-route to the hospital
A&E	I need emergency hospital treatment
Urgent Primary Care	I should be seen today, by a GP, urgent care centre, or similar
Routine GP	I should be seen at some point, but it can wait
Self-care	I can handle this at home or with over-the-counter medication

Participants are asked to identify the correct response to each scenario by selecting the best next step on a five-point scale modelled after entry points to the NHS. Explanations are provided to avoid differences in interpretation or idiosyncratic practical considerations.

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Extended Data Table 6 | Medical scenarios

Intended Condition	Scenario Excerpt	Best Disposition
Pulmonary Embolism	A 31-year old pregnant female has chest pain and shortness of breath	Ambulance
Pneumonia	A 42-year-old female who smokes wakes up in the night feeling very short of breath	Ambulance
Subarachnoid Haemorrhage	A 20-year-old male has a sudden and very severe headache	A&E
Renal Colic	A 47-year-old male has agonizing groin pain and bloody urine	A&E
Anaemia	A 26-year-old female feels constantly exhausted and nearly faints six months after giving birth	Urgent Primary Care
Ulcerative Collitis	A 24-year-old female has stomach pain, poor sleep and bloody diarrhoea	Urgent Primary Care
Tinnitus	A 46-year-old male is intermittently hearing a buzzing sound with no obvious source	Routine GP
Gallstones	A 36-year-old male has been feeling sudden sharp pain in the abdomen and is vomiting after eating fatty foods	Routine GP
Allergic Rhinitis	An 18-year-old male has itchy eyes, runny nose, and sneezing after working outside	Self-care
Common Cold	A 62-year-old female has a low fever and congestion	Self-care

A list of the medical scenarios used in the study. Each scenario was drafted and reviewed by a team of three practicing physicians, and proposed differential diagnoses were collected from an additional four physicians with expertise in general practice. As these are excerpts, the correct assessment may not be clear without additional context. For the complete scenario texts, see the Supplementary Materials.

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☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Data collection was conducted using the open source Dynabench platform (https://github.com/mlcommons/dynabench).
Data analysis	Data analysis code is available in the project repository (https://github.com/am-bean/HELPMed). Statistical tests were done in Python with statsmodels 0.14.3 and scipy v1.13.0.

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Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	We collect self-reported data on the sex of participants, which we use to stratify the experimental conditions. We had 677 female participants and 621 male participants. We report detailed breakdowns of the demographics and results by sex in the Supplementary Material.
Reporting on race, ethnicity, or other socially relevant groupings	We collect self-reported data on the ethnicity of participants, which we use to stratify the experimental conditions. We use five simplified categories based on the UK census, and target a representative sample. We had 1,072 white participants, 88 Asian participants, 49 Black participants, 42 Mixed participants, and 47 other participants. We report detailed demographic breakdowns in the Supplementary Material.
Population characteristics	See above
Recruitment	Participants were recruited via Prolific, which may lead to sampling biases as the population who take part in studies on Prolific must be online regularly and may also result in oversampling certain socioeconomic groups. This reduces the generalizability of the study, although people who are not regularly online are also unlikely to use language models for medical advice.
Ethics oversight	The study was approved by the departmental research ethics committee at the Oxford Internet Institute.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☒ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	*Describe how sample size was determined, detailing any statistical methods used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data exclusions	*Describe any data exclusions. If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Replication	*Describe the measures taken to verify the reproducibility of the experimental findings. If all attempts at replication were successful, confirm this OR if there are any findings that were not replicated or cannot be reproduced, note this and describe why.*
Randomization	*Describe how samples/organisms/participants were allocated into experimental groups. If allocation was not random, describe how covariates were controlled OR if this is not relevant to your study, explain why.*
Blinding	*Describe whether the investigators were blinded to group allocation during data collection and/or analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	The study compares four randomly assigned experimental conditions with quantitative experimental results.
Research sample	The sample is taken from the UK adult population using Prolific. We collected 2400 samples from 1298 adults. 1157 samples were collected from male participant, and 1253 samples were collected from female participants. 250 samples came from participants between 18-24 years old, 426 from participants 25-34 years old, 391 from participants 35-44 years old, 406 from participants 45-54 years old, 577 from participants 55-64 years old, and the remaining 350 from participants at least 65 years old. Using self-reported simplified ethnicity categories based on the UK census, 1982 samples were collected from White participants, 161 from Asian participants, 91 from Black participants, 77 from mixed ethnicity participants, and 89 from participants of other ethnicities. Each experimental condition is stratified to be representative of the general population based on the age, gender and ethnicity categories of the census. This sample enables the study to represent general public when interacting with LLMs.
Sampling strategy	Samples are stratified to ensure similar demographics between treatment groups. A pre-determined cutoff of 2400 observations was

Sampling strategy	determined based on budget constraints.
Data collection	The study was conducted online with form fields for participants to complete. The participants were blind to the experimental condition aside from the control group, which was able to identify that they were not given a language model to use. The researcher was not present during data collection, and was not blinded to the experimental conditions as a result.
Timing	Data collection ran between 4th September and 14th October 2024.
Data exclusions	We excluded data due to two technical issues. 1) An API issue caused participants in the treatment groups not to receive responses from the models. This led to the exclusion and replacement of 339 participants. 2) A recruitment platform issue allowed participants to complete the study more than once. This led to the exclusion and replacement of 21 participants.
Non-participation	520 participants began but did not complete the study. Of these, 395 dropped out before being assigned to a treatment group. The remaining 125 showed no association between treatment group and dropout rate (chi^2(3)=2.46, df=3, p=0.482).
Randomization	Participants were randomly allocated to the experimental conditions with stratified sampling to roughly match the covariates. The sample size was pre-determined to collect 300 interactions per experimental condition. A power analysis included in the Supplementary Materials showed this to be sufficient to capture a small effect with power of 0.90.

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.*
Research sample	*Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.*
Sampling strategy	*Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Reproducibility	*Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.*
Randomization	*Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.*
Blinding	*Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Did the study involve field work? ☐ Yes ☐ No

Field work, collection and transport

Field conditions	*Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).*
Location	*State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).*
Access & import/export	*Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).*
Disturbance	*Describe any disturbance caused by the study and how it was minimized.*

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used — *Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.*

Validation — *Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.*

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s) — *State the source of each cell line used and the sex of all primary cell lines and cells derived from human participants or vertebrate models.*

Authentication — *Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.*

Mycoplasma contamination — *Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.*

Commonly misidentified lines (See ICLAC register) — *Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Palaeontology and Archaeology

Specimen provenance — *Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.*

Specimen deposition — *Indicate where the specimens have been deposited to permit free access by other researchers.*

Dating methods — *If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight — *Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals — *For laboratory animals, report species, strain and age OR state that the study did not involve laboratory animals.*

Wild animals	*Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*
Reporting on sex	*Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.*
Field-collected samples	*For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*
Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☐	☐	Public health
☐	☐	National security
☐	☐	Crops and/or livestock
☐	☐	Ecosystems
☐	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☐	☐	Demonstrate how to render a vaccine ineffective
☐	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☐	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☐	☐	Increase transmissibility of a pathogen
☐	☐	Alter the host range of a pathogen
☐	☐	Enable evasion of diagnostic/detection modalities
☐	☐	Enable the weaponization of a biological agent or toxin
☐	☐	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks: N/A

Novel plant genotypes: N/A

Authentication: N/A

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links
May remain private before publication.

For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission

Provide a list of all files available in the database submission.

Genome browser session
(e.g. UCSC)

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates

Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth

Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

Antibodies

Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters

Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.

Data quality

Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software

Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.

Instrument

Identify the instrument used for data collection, specifying make and model number.

Software

Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.

Cell population abundance

Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.

Gating strategy

Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template

Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

Noise and artifact removal

Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

(See Eklund et al. 2016)

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.