Canis familiaris as model for non-invasive comparative neuroscience

Nóra Bunford[1*], Attila Andics[1,2], Anna Kis[3], Ádám Miklósi[1,2], and Márta Gácsi[1,2]

[1] Eötvös Loránd University, Institute of Biology, Department of Ethology, 1117 Budapest, Pázmány Péter sétány 1/C
[2] MTA-ELTE Comparative Ethology Research Group, 1117 Budapest, Pázmány Péter sétány 1/C
[3] Institute of Cognitive Neuroscience and Psychology, Hungarian Academy of Sciences, 1117 Budapest, Magyar tudósok krt 2.

*Correspondence: bunfordnora@caesar.elte.hu (N. Bunford).

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Abstract: There is ongoing need to identify and improve animal models of human behaviour and biological underpinnings thereof. The domestic dog (*Canis familiaris*) is a promising model in cognitive neuroscience. However, before it can contribute to advancements in such science in a relevantly comparative, reliable, and valid manner, methodological questions warrant attention. To base the research on rigorous foundations, we review non-invasive canine neuroscience studies, primarily focusing on 1) variability across dogs and between dogs and humans in cranial characteristics and 2) generalizability across dog and dog-human studies. Arguing not for methodological uniformity but for *functional comparability* in study methods, experimental design, and neural responses, we conclude that the dog may become an innovative and unique model in comparative cognitive neuroscience, one that is complementary to traditional models.

Animal models in comparative neuroscience

Animal model research is grounded in the idea that animals share behavioural, physiological, and other characteristics with humans. Benefits of such research include increased understanding of phenomena that could not be directly studied in humans or without cross-species comparison. The neuroscience of socio-cognition has been extended from traditional primate and rodent models to the domestic dog – an alternative, complementary model that permits for non-invasive measurement of behaviour and its neural correlates. There has been an upsurge in canine neuroscience studies, necessitating establishment of methodological guidelines that ensure scientific rigor. To this end, complementing available reviews that are heavily [1] or solely [2] focused on available fMRI findings [1,2] from a conceptual perspective, we review the non-invasive canine neuroscience literature, focusing on methodology and experimental design. Primarily guided by principles of comparative anatomy, we highlight advantages of and remaining challenges of the dog as an animal model for comparative cognitive neuroscience.

We begin with an overview of animal models of human behaviour, then narrow our focus into neuroscience, leading to questions about the domestic dog as a model for comparative neuroscience. Mainly focusing on non-invasive canine fMRI and EEG research, we reflect on such questions in light of three main considerations. These centre on within- and between-species variability, in particular in cranial characteristics, though are also varied in terms of the degree to which they potentiate (1) advantages and disadvantages for the dog as an animal model and, in case of disadvantages, whether solutions (2) have or (3) have not been developed to address those.

Animal models for comparative cognitive neuroscience

A goal of comparative research is to establish principles of **proximate and ultimate causation** (see Glossary), via between-species comparisons and study of individual organisms. Animal models for comparative cognitive science include avian [3–5] as well as rodent and primate models that have emerged as primary models for comparative cognitive *neuro*science [2]. Advantages of rodents include feasibility of handling the animals under laboratory conditions; cost-efficiency; and utility in pre-clinical

and clinical studies [6]. Advantages of primates include similarity to humans in development, neuroanatomy, physiology, and reproduction, as well as in cognition and social complexity and thus suitability for studying a range of mental processes [7]. Yet, use of these models is increasingly problematic for animal welfare and ethical reasons [8]. Conversely, the role of the domestic dog has been becoming increasingly important, with research initially focused on informing treatment for human medical diseases with laboratory dogs [e.g., 4] and more recently involving basic research on sensation, perception, and socio-cognition with family dogs (Box 1). One reason for this increase in importance is that dogs, having been encultured in human society, naturally exhibit *cooperativeness* and *trainability, obviating need for fluid and/or food restriction as a motivational tool.* Thus, relative to other species, preparation of the dog for an experiment is more similar to preparation of humans in terms of corresponding physiological and social state and there is less limitation to generalizability of interaction with experimenters and environmental (e.g., lighting and sound) and experimental stimuli [1]. *Cooperativeness and trainability* also *permit for non-invasive methods;* although techniques have been developed for awake scanning of monkeys, pigeons, and rats [1], unlike these animals but like humans, dogs do not need to be restrained (e.g., via surgically implanted posts [10]) but can be trained to hold still, yielding more valid cross-species comparisons. Finally, given their evolutionary history and integration with humans, dogs and humans exhibit a range of socio-cognitive skills that share key behavioural and functional characteristics [11]. It is for ability to study these very skills and corresponding functions (Box 1) that the dog may be one of the best model species for study of human socio-cognition [2] in *comparative* neuroscience [11].

Together, it stands to reason that the domestic dog is a suitable model for comparative neuroscience and that the non-invasive methods of brain circuits, physiology, and behaviour used with the dog ideally complement the invasive methods appropriate for studying molecules and cells used with traditional models. In combination with over 20 years of canine ethological research [12] and capitalizing on exciting possibilities of the species and non-invasive methods, there has been an increase in the number of

canine neuroscience studies, with an overwhelming majority conducted in the past 3 years. (Mostly fMRI and EEG, although other methods have also been used [13]).

Basic standards for measures and methods include reliability and validity [14] and, in case of comparative research, for them also to be *relevantly* comparative. Related pressing questions pertain to the degree to which methods are comparable across dog-dog and dog-human studies as well as the degree to which employed methods allow for comparability and generalizability across studies (Table 1, Key Table); with the impetus behind such questions stemming from within- and between-species variability, especially in cranial characteristics. Some of this variability presents advantages for the dog as a model and some may be limiting. In the latter cases, methods to address limitations are either already being developed and evaluated or are in need of development and evaluation.

Differences that present advantages

Differences in skull formation and brain anatomy. Across humans, variation in skull formation and brain size is relatively trivial; the average female brain volume is 90% of the male [15] and the average brain volume of a 7-11-year old child is 95% of the volume of a sex-matched adult [16]. Conversely, there are large differences across dogs in skull shape and size and brain anatomy. Canine skull length ranges from 7 to 28 cms [17] (i.e., the shortest dog skull is 25% of the longest), making *Canis familiaris* the species with most within-species morphological variation in this regard [18].

In addition to skull length, differences across **dolichocephalic, brachycephalic**, and **mesaticephalic** dogs include dissimilarities in the craniofacial angle (angle between the **basilar axis** and **hard palate**) [19], in neuroanatomy (e.g., in brachycephalic dogs the brain is rotated with respect to its mediolateral axis) and the anatomy of the cerebral cortex [20], temporomandibular joint (i.e., jaw joint) [21], and **cribriform plate** [22].

These differences across dogs *allow for examining the relation among brain structure, function, and behaviour within the same species* and the effects of differences in skull- and brain-morphology on neuro-socio-cognition. As the ≥400 documented breeds exhibit a variety of genetically fixed morphologic traits that correspond to differences in behaviour, longevity, size, skull shape, and disease susceptibility

[20], better understanding of these was proposed to increase understanding of mammalian biological and embryonic development [20]. Although, to date, the number of dog breeds involved in fMRI studies is considerably lower, they include subjects from diverse breeds suggesting that there is no limitation (e.g. in trainability) to between-breed comparisons.

In support of stated advantages, differences in dog skull shape are associated with differences in brain organization, e.g., brachycephalic brains are relatively rounded and shortened in the anterior-posterior plane, the brain pitched ventrally at the anterior pole, with a pronounced shift in the position of the olfactory lobe [18] (see Box 2 for additional examples). Differences in skull shape are further associated with differences in behaviour in that brachycephaly, relative to dolichocephaly, is associated with increased ability to focus and rely on human gestures [23]. Conversely, less morphological differences across individuals in other species, such as humans, are less (or not) suitable for addressing these questions and are thus largely overlooked.

Differences in experimental design: sample composition. Compared to the human neuroscience literature, there is significant overlap in groups of dogs across studies. This is due, in part, to challenges (e.g., limited subject availability and need for extensive training) and, in part, to advantages that make the dog a multi-experiment model (e.g., ability to re-measure dogs as they do not need to be euthanized after participation). For example, in canine fMRI studies, 100% of the sample of [24] was included in [25], and there was a 92% overlap in the samples of [25] and [26], and a 67% overlap in the samples of [25] and [27], and all dogs in [28] came from one of these samples. Similarly, in EEG studies, there was a 100% overlap in the samples of [29] and [30], and a 68% overlap in the samples of [31] and [32].

Awake fMRI testing necessitates that dogs are trained to get used to scanner coil; place their heads in-between their paws [34,35,37–39] or on a chinrest [24–28,33,36,40], and hold this position until a release signal and then while wearing canine ear muffs; get used to recordings of scanner noise and being in a mock scanner; and to adhere to these procedures inside the scanner room and ultimately the scanner [25,34]. Training is extensive and typically involves behavioural shaping, conditioning and social learning (e.g., the "**Model/Rival" training method** [34]). Different training methods allow for different

lengths of time during which dogs are able to hold a position, which has implications for design. For example, in some studies, consistent with human studies, dogs do not exit the scanner between runs [34,35,37,39,41] whereas in others, they do [24–28,33,36,40]. Movement artefacts are also handled differently: some authors, consistent with human studies, exclude scans with head translation >3mm or rotation $>1^0$ [34–36]; whereas others exclude scans with >1% scan-to-scan signal change [24]; >0.1 fraction of outlier voxels in each volume or >1% scan-to-scan signal change, in combination with >1mm scan-to-scan displacement [26–28,40,42], and yet others exclude runs with .10mm total displacement [37,41]. As the size of the dog brain is roughly one-third of the human, arguably, a >3mm translation in dogs would approximate an unacceptable >9mm in humans. However, in most studies where the human criteria were used, translation did not exceed 1mm [34,43]. Additionally, it has been shown that changes in the time course of fMRI data are decreased when correlations are examined long-distance but increased when they are examined short-distance, indicating that absolute movement is less and relative movement is more important when pre-processing the data [44]. Finally, depending on study design and research group, dogs need anywhere from five sessions [34] to 18 months of training [25]. For comparison, human adults do not receive training and human children as young as 6 years of age receive minimal (a one-, maximum two-occasion, 30-60-minute familiarization with a mock-scanner and recordings of scanner noise) or no training [45] (Table 1).

The overlap in groups of dogs included across studies also has advantages for examination of reliability and validity of measures as it allows for assessment of *within-subject stability vs. change of measures of neural function over time* and of *within-subject correspondence of neural correlates and performance across social, cognitive, and affective paradigms*. This ability to examine psychometric properties of measures is comparable to research with humans but not most other species, where animals easily habituate or are euthanized following participation. Regarding *within-subject stability vs. change over time*, although the reliability, including test-retest reliability, of neuroimaging [14] has, until recently, been a relatively neglected area of research in human neuroscience, the overlap in groups of dogs across canine studies presents a natural opportunity to attend to questions of psychometrics [46].

Regarding *within-subject correspondence of neural correlates and performance across paradigms*, it is important that these exhibit convergence and divergence, where expected. Establishing correspondence across different indices of phenomena of interest (e.g., social and cognitive indices of self-regulation) but that these provide unique information about variables examined, is key to the innovative dimensional frameworks that are currently championed (e.g., the Research Domain Criteria [RDoC]; [47]).

Differences that potentiate disadvantages but solutions are available

Within-species differences in skull formation and brain anatomy. Thess within-species variabilities (Figure 1) are relevant for normalization. In fMRI research, advantages of normalization are that when a set of coordinates is referenced, the location to which those coordinates correspond is known and that results can be: generalized to a larger population; compared across studies wherein the same brain is used for normalization; and can be averaged across subjects for group-level analyses. Disadvantages are that it reduces spatial resolution and increases probability of error in identification of anatomical location.

Normalization requires a "standard" brain, i.e., template. In the adult human literature, the Montreal Neurological Institute (MNI) template (MNI305) is commonly used (Table 1), which is based on combination of 152 healthy adult MRI scans [48]. Given relatively little difference between adult and child brains, the MNI-305 is suitable for use with children over age 6 years [49] and empirical studies have generally followed suit, with some attempts at developing a child template for use with a wider range of ages (e.g., from 2 weeks to 4.3 years [50] and 4.5 years through 19.5 years (on age increments of 6 months [51]). Conversely, at present, there is no widely-accepted and used dog template. Authors of canine fMRI studies have addressed this issue by omitting group-level analyses altogether or, where group-level analyses were conducted, by using the brain of a selected individual, or using a template based on the brains of 15 mesaticephalic dogs (Table 1).

Besides the said advantages of population-based templates, there are advantages of study-specific templates [52] (a special case of which is use of the brain of a selected individual). Regarding the Datta

193 atlas [53], one limitation is that head length and width may influence cortical folding in a manner that an
194 affine transformation of brain size may not correct for, indicating that the Datta template may not be
195 appropriate for non-mesaticephalic animals.

196 Challenges resulting from within-species differences in skull formation and brain anatomy across
197 dogs have been addressed differently in canine continuous EEG and in event-related potential (ERP)
198 studies. Regarding **continuous EEG**, presumably due to differences in skull morphology (e.g., thickness
199 of the frontal and parietal bones), absolute EEG power (μV^2) varies greatly across dogs (e.g., 3-fold
200 across our samples; [31,32]). As a result, group-level analyses are best conducted using relative EEG
201 spectrum values [31,32], which is common practice in human EEG studies as absolute EEG power is less
202 psychometrically sound than relative EEG power. Regarding **ERP** research, challenges have been
203 addressed either via use of a homogenous group of dogs (e.g., laboratory-bred and -kept beagles, all of the
204 same age and similar weight [29,30]) or via report of results at the level of individual dogs [54].

205 Relevant for both continuous EEG and ERP studies, an additional methodological issue is electrode
206 placement. Despite canine methods having been adopted from human studies, given variability in dog
207 head shape and size, the distance between electrodes placed on anatomical landmarks is different across
208 dogs. Although this difference is difficult to address, such variation in absolute distances are compatible
209 with the **International 10-20 system** used in human studies [55], which keeps not the absolute but the
210 relative distance between electrodes constant.

211 ***Between-species differences in skull formation and brain anatomy*** (Box 2; Figure 1). In fMRI,
212 these differences highlight consideration related to correction for multiple comparisons (Box 3). Given
213 smaller brain volume of dogs relative to humans, the multiple comparison problem is less relevant in
214 canine fMRI. If correction that takes voxel number into account is used in a human and a dog study or
215 across dog studies, results are comparable. If correction that does not take such number into account is
216 used, it is important that the search area is comparable in size. Both are feasible. Nevertheless, although
217 there are widely used methods for correction in human studies and these are now employed in most (if not
218 all) adult and child studies [56], there is heterogeneity across dog studies (Table 1). No meaningful

comparison can be made between results obtained without and with correction, with varying degrees of stringency. If and when the aim is to compare results, consistency across studies will be important.

In EEG research, differences between dog and human skull and brain morphology necessitate differences in electrode placement. Because dogs have a smaller but more muscular head than humans, their heads permit less sites for electrode placement. The number of electrode holders in human EEG head caps range from 16 to 256 compared to 3 [54,57], 4 [32], or 5-7 [29–31] electrodes placed on dogs' heads. Nevertheless, as these sites correspond to human electrode sites, a *functional comparison* between species can be made, even if restricted to a small number of EEG channels, which may be further increased with methodological advancements.

Differences in experimental design: sample composition. Available findings having been obtained with a small group of dogs and the noted overlap in included dogs may be disadvantageous for generalizability to larger dog populations. This can be addressed through sample selection that increases generalizability potential, e.g., ensuring that dogs of different ages, breeds, sexes, and level of prior training (e.g., from training-naïve to service dogs), are included and then tested. Selection of a biologically and demographically heterogeneous sample with variation in training history has been attended to with varying degrees, with some variability in laboratory [29,30,37,39,41] vs. family [24–28,31–36,40,54,57] dogs, single [29,30,37,39,41,57] vs. multiple [24–27,31,32,34–36,40,54] breeds (with [28,33] not specified), and ages ranging from 1 to 12 years.

The noted small sample sizes and overlap in included dogs also means a very small overall number of tested dogs. The sample sizes of all but one [32] canine neuroscience studies published to date are <15, leaving the research underpowered and effects difficult to detect. Although the obtained results may reflect effects that are so large and robust that they are detectable even with small samples, they may alternatively reflect effects that are fragile, non-generalizable, or spurious. Power analysis indicates that larger samples are needed for confidence in results [58]. Yet, it is also the case that in early and exploratory stages of a research area, small *N* studies are not only warranted but also desired to establish that larger (necessitating more funds and participant and researcher time) studies are indicated.

Differences that potentiate disadvantages and solution need to be identified

Between-species differences in skull formation, brain anatomy, and physiology. Although further research is needed about *the degree to which dogs' anatomical structures and circuits correspond to humans',* knowledge about canine brain anatomy and the similarities between such anatomy and that of humans' is encouraging regarding the dog as an animal model in comparative neuroscience. There is evidence of correspondence between the species in, for example, primary sensory areas and associated functions [34]. Yet, whether other areas, especially the frontal and prefrontal cortex are organized in a manner that allows for characterization of structures and circuits as associated with similar cognitive functions across dogs and humans is largely unknown. As such, when a specific human structure is referenced (e.g., rostral anterior cingulate cortex [rACC] or dorsolateral prefrontal cortex [DLPFC]), it is, at present, unclear whether the rACC in dogs is anatomically delineable from other areas of the ACC *and* functionally (e.g., attentional control over emotional conflict or distracters [46,59]) the same or at least meaningfully comparable across the species.

The solution to this challenge is unclear as from a biological perspective, there is no "reference species" that is uniformly appropriate for addressing pertinent questions. Would it be prudent to take rodents as a reference? Although rodent brains are more dissimilar from human brains than dog brains, evidence obtained via invasive methods indicates correspondence in certain structures across rodents and humans [60]. Alternatively, would it be useful to take humans as a reference and identify areas of activation to stimuli, present dogs with comparable stimuli and search for correspondence in the canine brain? Then again, in addition to or instead, is there need for research that identifies parallels through ontogeny? For example, although there are differences between birds and apes in neural structures, e.g., birds do not have a cerebral cortex for processing complex mental tasks [5], both species have prefrontal structures that control comparable executive functions [5]. It has been argued that these similarities either originated from the last common ancestor passing down neuronal bases of executive functions or evolved independently due to the species facing similar challenges [5].

Between-species differences in skull formation and brain anatomy are also source of methodological shortcomings in fMRI as the obtained images are of poor quality due to use of radiofrequency (RF) coils (human head/neck coils [24–28,33] or knee coils [34–39]) whose geometries have been optimized for different purposes and have not been tailored to dogs' heads and neuroanatomy, making them less than ideal for canine fMRI. Together, as was the case with other species (e.g., marmosets, rats, mice, and rhesus monkeys) where use of dedicated animal coils has been shown to improve signal-to-noise ratio (SNR) [10], there is need for development of dedicated dog coils that satisfy the anatomical constraints imposed by these animals. Until such coils are available, it will be important for research to determine which coil type is best for performing fMRI in awake dogs with sensitivity, specificity, and large functional contrast-to-noise ratio [1].

Between-species differences in cranial musculature and size are relevant for artefact rejection in EEG (Box 4). In human studies, artefact rejection includes correction for ocular artefacts and quantitative procedures (e.g., removing artefacts with voltage step between sample points that is greater than e.g., 50μV; with voltage difference of e.g., 300μV within a trial; and maximum voltage difference within e.g., 100msec intervals of e.g., <0.5μV [61]) and rejection via visual inspection. In dog studies, there are no well-established quantitative procedures, given difficulty in distinguishing muscle artefact from EEG signal and artefact rejection is typically done using simpler methods. The authors of ERP studies used only a single crude method [62] for rejecting trials with artefacts, in which a trial is rejected if the voltage during the epoch exceeds a user-defined threshold (amplitudes higher than 100μV [54,57] or 200μV [29,30]) and the authors of sleep EEG studies conduct artefact rejection by visual inspection only [31,32].

Although the user-defined method works for rejection of artefacts resulting from blinks, it is inadequate for detecting more subtle artefacts, such as those resulting from eye (or ear) movements [62]. As such, the used methods are problematic for awake continuous EEG measurement and ERP data collection where there is need for more stringent artefact rejection, given greater canine cranial muscle mass; another example where methodological uniformity between human and dog studies is neither possible, nor warranted. As an example, if the dog moves its eye (or ear) every time there is an event (i.e.,

296 stimulus), it is difficult to determine whether what appears to be a voltage change reflects the movement
297 or differential neural activation. It may be for this reason that there are no established methods for non-
298 invasive measurement of ERPs in dogs, albeit some non- [29,30] and semi-invasive studies suggest
299 progress [42,44].

300 Potential solutions to the artefact problem in non-invasive canine ERP research is to collect data
301 from dogs with less cranial muscle and/or in a state of drowsiness (i.e., canine equivalent of light sleep) or
302 sleep. In support, the Mismatch Negativity (MMN) component can be elicited during light sleep in
303 humans [63,64], indicating an auditory ERP method may be useable with drowsy dogs. Notably, dogs
304 spend at least 30 minutes in drowsiness during a 3-hour-long spontaneous EEG recording [32]. Not unlike
305 sleep, drowsiness is characterized by lowered muscle tone, indicating it permits a considerable amount of
306 artefact-free EEG data that ERP studies could potentially capitalize upon.

307 Between-species differences are pertinent beyond skull formation, brain anatomy and include
308 differences in resting state physiology. Specifically, normal respiratory rate in newborn puppies may be as
309 low as 15 breaths/minute and in an average adult dog it is 24 breaths/min [65]. Conversely, respiratory
310 rate in human neonates (<1 year old) is 30-40 breaths/min, in older children/young adolescents (5-12-
311 year-olds) it is 20-25 breaths/min [66] and in a healthy adult it is 12–20 breaths/min [67]. With regard to
312 heart rate, <2-week-old puppies have 160-200 beats/min (bpm), ≥2-week-old puppies have up to 220
313 bpm, and adult dogs have 60-140 bpm [65,68]. For comparison, human neonates (<1 year old) have 110-
314 160 bpm and older children and young adolescents (5-12-year-olds) have 80-120 bpm [66]. The heart rate
315 of a healthy adult is between 50–90 bpm [69].

316 These between-species differences are important as differences in brain shape and size also results
317 in between-species differences in the hemodynamic response function (i.e., the course of the
318 hemodynamic response to an external stimulus – the most common functional imaging signal; HRF) [1]
319 and respiratory rate and heart rate are major sources of fMRI confounds as they are correlated with
320 changes in BOLD signal [70]. The shape of the canine HRF is currently unknown [1] potentially due to
321 the temporal resolution in canine fMRI studies, where repetition time (TR) varies between 1-2secs, which

is insufficient to sample respiratory or heart rate in dogs. Related, the number of acquired datasets is limited by how long dogs are able to hold still (with experiments necessitating 5- [24], 6- [27,34,40], 7.5- [35], 10- [71], and some 14-minute-runs [26]) (no information is provided in [28,36,39]). As such, the measurement duration that maximizes data quality is unknown. To identify an optimal parameter setup, different anatomical and functional sequence parameters should be tested with phantom and ex-vivo measurements. Similarly, protocols should be optimized with respect to signal- and contrast-to-noise ratio in pilot samples sufficiently similar to the intended experimental samples, but without the constraints on measurement time and motion of in vivo measurements. The ultimate goal of adapting sequence parameters to the dog brain is combination of high spatial and high temporal resolution. Such adaptation will have account for the smaller size of the dog brain, differences in dog compared to human physiology, and limits on run length by how long dogs are able to hold still. Importantly, there are methodological and ethical advantages to shorter runs as these minimize image deterioration due to motion artefacts and prevent rises in specific absorption rates (SAR) of radio frequency levels (see *Ethics and Safety*) [1].

Differences in skull formation and brain anatomy: within- and between-species. Combined, differences across dogs and between dogs and humans in cranial characteristics will make it difficult to determine whether measured electrocortical signal originates from a meaningfully comparable population of neurons across dogs and dogs and humans. Even the human source localisation literature is in its early stages, with only a few studies on the association between BOLD signal and ERPs recorded during the same session [72]. As the human literature advances, it will be important for canine research to make parallel progress. As noted, little is known about the degree to which certain neural structures in dogs are anatomically *and* functionally the same as humans' and advancing the literature in this domain will also be important for source localization.

Differences in experimental design: active vs. passive paradigm. In the human neuroscience literature, there are examples of studies where no behavioural response is required (passive task) and where a response is required (active task). From the perspective of introducing additional movement that results in additional motion artefact, as passive tasks do not involve movement, they are not problematic.

In humans, active tasks are also feasible with behavioural responses like a button press. In dogs, requiring an active response would mean that images obtained following an active condition have to be discarded.

Indeed, in all but one of canine fMRI studies, the functions that have been examined are ones that do not necessitate an active response, including in *passive auditory paradigms* [34,35], *passive visual paradigms* [24,27,28,36,42], *passive olfactory paradigms* [37] or, finally, probing resting state activity. In the only canine fMRI study, with an active, go/no-go paradigm, a "go" signal indicated an active behavioural response is to be executed, which, in this case involved dogs touching a target with their noses while in the scanner. When analysing human go/no-go data, go trials are typically compared to no-go trials [73]. Here, however, activation during inhibition trials was compared with activation during neutral trials as successful "go" trials could not be analysed due to the head motion produced by the nose-touch. This is an important limitation to the current state of the canine neuroscience field as there are socio-cognitive functions that are best probed in active paradigms.

In addition, the likelihood of prematurely attributing connections between brain structure and function is enhanced when the aim is to separate active and passive processing in dogs, as in the absence of concurrent behavioural response, the relevant cognitive processes are unknown. Being able to differentiate between active and passive processing in dogs will be key, as there are differences in activation to these two forms of processing in humans. One solution to ameliorate risk of reverse inference (i.e., *post hoc* attribution of presence of a certain cognitive process given activation) is ensuring that dogs have pre-fMRI training on a behavioural paradigm that probes the same cognitive process the fMRI task in question is intended to probe [1] (see, for example, [27]). On a related note, as discussed in relation to the overlap in groups of dogs included across studies, the most ideal assessment battery will comprise measurement methods representing different levels of the measurement continuum (ranging from micro level measurement of brain circuits via fMRI, through less micro level measurement of physiology through EEG, to macro level measurement of observable behaviour via observation or rating scales; [74]) as data obtained at these different levels provide unique information on characteristics of interest [46,61,75–77].

Ethics and safety

As noted, a main advantage of dogs is that being a domestic animal they can be tested without need for laboratory breeding, raising and keeping. As such, focus on family dogs is what makes the advantage of the dog model ethically permissible. Nevertheless, as aptly discussed by others [1], care should be exercised that no harm is caused, e.g., that scanner noise and high sound pressure levels do not lead to discomfort and hearing damage or that specific absorption rates (SAR) of radio frequencies do not reach harmful levels of rise in tissue temperature [1].

During tests, dogs' well-being should be continuously monitored and undue stress eliminated both for reasons of ethics and because stress can lead to increases in physiological activity such as increased respiration and tachycardia, which, as noted, may introduce non-neural noise. The techniques used by canine neuroscience laboratories address stress reduction via use of sound-attenuating earmuffs and in training [1]. Stress reduction can be further improved through careful selection of sequence parameters combined with pre- and post-scanning measurement of physiological indices (e.g., cortisol) of stress such as from saliva or urine [1]. SAR should be measured throughout MR scans and in the absence of established guidelines for nonhuman animals, researchers may adhere to standards established for humans.

Concluding remarks

There has been a notable, recent increase in canine neuroscience studies, necessitating establishment of methodological guidelines and standardisation to inform the next generation of studies in the area. We discussed foremost questions related to methodology and experimental design in the canine neuroscience literature. As a result, we identified areas for further empirical inquiry. Capitalizing on advantages of the dog such as its cooperativeness and trainability, further areas of exploration include the relation among brain structure, function, and behaviour in dogs, within-subject temporal stability of neural measures, and within-subject correspondence of neural correlates. In addition, we suggest to evaluate and performance across social, cognitive, and affective paradigms, in particular probing socio-cognitive skills that share key behavioural and functional characteristics across dogs and humans.

Regarding challenges for which solutions are already being employed, it will be important that such solutions are adopted and used in a reasonably standardised fashion. Regarding unresolved challenges, it will be important to ensure that samples of dogs reflect variation in the larger population to increase generalizability. Specific to fMRI, it will be key to improve sensitivity of imaging protocols and image quality including via improved spatial and temporal resolution that also allow for sampling heart and respiratory rate as well as development of sequence parameters and dog coils and that are tailored to the specifics of dogs and their neuroanatomy. It is unknown whether non-invasive ERP research is possible with dogs. Addressing this question may necessitate more sophisticated methods either for minimizing eye-movement and muscle artefact during experiments and/or for artefact rejection (e.g., filtering) that is appropriate to the magnitude and type of artefact that occurs in dogs. The degree to which neural structures in dogs are anatomically *and* functionally comparable to those of humans will need to be established, including to set the stage for future studies with simultaneous neuroimaging and electrophysiological measurement aimed at source localisation. Source localisation will, in turn, help uncover the degree to which what appears to be meaningfully comparable electrode placement across dogs (and across dogs and humans) reflects signal from a meaningfully comparable population of neurons. Regarding difficulty with active behavioural paradigms, methods need to be identified that either permit for dogs to exhibit a behavioural response without data loss or, alternatively, passive paradigms that probe functions that currently can only be manipulated in active paradigms need to be developed.

In closing, we argue that, carefully considering inherent advantages, the domestic dog may become an innovative and unique model for comparative cognitive neuroscience. This becomes relevant if the highlighted advancements take place as these will be necessary for measuring the neural bases of canine socio-cognition in a relevantly comparative, reliable, and valid manner. Addressing the noted challenges with dogs appears appreciably more feasible than addressing those with traditional models, such as their non-cooperativeness, them not sharing a social environment with humans, and, in case of primates, cost-inefficiency and paucity.

Glossary

Basilar axis: the axis corresponding to the base of the skull
Bradicephalic: short skulled
Calvaria: the bone that covers the cranial cavity containing the brain, i.e., the skullcap
Continuous EEG: continuous measurement of electrocortical signal, i.e., not measurement of change in such signal in response to a stimulus
Cribriform plate: a structure that forms the caudal boundary of the nasal cavity
Dolichocephalic: long skulled
ERP: measurement of negative and positive voltage changes in electrocortical signal in response to specific events (e.g., stimuli)
Gyrencephalic brain: with brain folds (gyri) and grooves (sulci), i.e., folded brain
Hard palate: a thin horizontal bony plate of the skull, located in the roof of the mouth
Homology: shared ancestry between a pair of genes or structures, in different taxa. A common example is the vertebrate forelimb, where bat wings, primate arms, whale front flippers, and dog forelegs are all derived from the same ancestral tetrapod structure. The opposite of homologous genes or structures are analogous ones, i.e., ones that serve a similar function across two taxa but were not present in their last common ancestor but evolved independently. For example, the wings of a bird and a sycamore maple seed are analogous (but not homologous), as they developed from different structures.
International 10–20 system: a method used to describe the location- and guide the application of scalp electrodes in an EEG examination or experiment, based on the relation between placement of an electrode and underlying cortex. The 10-20 system was developed to ensure reproducibility and standardisation. The "10" and "20" refer to the distances between adjacent electrodes being 10% and 20% of the total front–back or right–left distance of the skull, respectively.
Lissencephalic brain: without brain folds (gyri) and grooves (sulci), i.e., smooth brain
Mesaticephalic: a mesaticephalic skull is neither markedly dolichocephalic or brachycephalic and is of intermediate length and width
Model/Rival method: a social learning training method where during the training of an individual, another individual can be present and when the model is rewarded and praised for the wanted behaviour the rival is ignored
Prehensile organ: an organ adapted for seizing or grasping especially by wrapping around
Proximate causation: an explanation of biological functions and traits in terms of the effects of immediate environmental forces
Somatotopic organization: various portions of the body are represented topographically on specific regions of the cerebral gyri
Somesthetic cerebral cortex: the primary cortical processing mechanism for sensory information originating at the body-surfaces (e.g., touch) and in deeper tissues such as muscle, tendons, and joint capsules (i.e., position sense).
Ultimate causation: an explanation of biological functions and traits in terms of the effects of evolutionary forces

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