ORIGINAL INVESTIGATION

Differential effects of the D- and L- isomers of amphetamine on pharmacological MRI BOLD contrast in the rat

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Abstract

Rationale The D- and L-amphetamine sulphate isomers are used in the formulation of Adderall XR®, which is effective in the treatment of attention-deficit hyperactivity disorder (ADHD). The effects of these isomers on brain activity has not been examined using neuroimaging.

Objectives This study determines the pharmacological magnetic resonance imaging blood-oxygenation-level-dependent (BOLD) response in rat brain regions after administration of each isomer.

Materials and methods Rats were individually placed into a 2.35 T Bruker magnet for 60 min to achieve basal recording of variation in signal intensity. Either saline ($n=9$), D-amphetamine sulphate (2 mg/kg, i.p.; $n=9$) or L-amphetamine sulphate (4 mg/kg, i.p.; $n=9$) were administered, and recording continued for a further 90 min. Data were analysed for BOLD effects using statistical parametric maps. Blood pressure, blood gases and respiratory rate were monitored during scanning.

Results The isomers show overlapping effects on the BOLD responses in areas including nucleus accumbens, medial entorhinal cortex, colliculi, field CA1 of hippocampus and thalamic nuclei. The L-isomer produced greater global changes in the positive BOLD response than the D-isomer, including the somatosensory and motor cortices and frontal brain regions such as the orbitofrontal cortices, prelimbic and infralimbic cortex which were not observed with the D-isomer.

Conclusions The amphetamine isomers produce different BOLD responses in brain areas related to cognition, pleasure, pain processing and motor control probably because of variations on brain amine systems such as dopamine and noradrenaline. The isomers may, therefore, have distinct actions on brain regions affected in ADHD patients.

Keywords ADHD · Amphetamine · fMRI · phMRI · BOLD · Striatum · Prefrontal cortex

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Introduction

Amphetamines were developed in the 1950s and 1960s and, more recently, have been included as components of Adderall XR®, a drug used in the treatment of attention-deficit hyperactivity disorder (ADHD). Adderall XR® is a mixture of D-amphetamine sulphate, L-amphetamine sulphate, D-amphetamine saccharate, and D-, L-amphetamine aspartate. For each Adderall XR® tablet, the combination of salts and isomers results in a 3:1 ratio of dextroamphetamine/levoamphetamine. Microdialysis and in vitro studies have consistently demonstrated stereoselective effects of the D- and L-isomers of amphetamine on the uptake, release and extracellular levels of monamines. The D-isomer is reported to have around twice the potency of the L-isomer on dopamine reuptake and release in the striatum (Coyle and Snyder 1969; Ferris et al. 1972; Harris and Baldessarini 1973; Heikkila et al. 1975; Holmes and Rutledge 1976). This stereospecific difference also occurs with noradrenaline release in the cortex where the L-isomer is equipotent (Heikkila et al. 1975) or more potent (Easton et al. 2007b)

than the D-isomer. Furthermore, stereoselective effects of the isomers on behavioural measures have been demonstrated in rodents where the D-isomer increases locomotor activity and the duration of stereotypy more than the L-isomer (Segal 1975; Kuczenski et al. 1995). As a balance of dopamine versus noradrenaline activity is considered critical for ADHD treatment (Rogeness et al. 1992) and for normal frontal lobe function in attention, working memory and behavioural inhibition (Arnsten and Li 2005), there is the possibility that each amphetamine isomer may produce different neuronal effects in frontal areas of the brain.

Pharmacological magnetic resonance imaging (phMRI) evidence also shows that atomoxetine and guanfacine, which are also effective treatments for ADHD, show similar effects on the negative blood-oxygen-level-dependent (BOLD) response in caudal areas of the brain and exert effects on cortico-basal thalamic loop circuits (Easton et al. 2006, 2007a). The effect of amphetamine on the phMRI BOLD response has been examined in rats (Dixon et al. 2005), and although it was not clarified within this manuscript, it utilised the D-isomer (personal communication). The phMRI BOLD response has also been used to show how D-amphetamine modulates the efficiency of verbal working memory (Tipper et al. 2005) and produces changes in neuronal activity in humans undergoing cognitive tasks (Willson et al. 2004). A more recent study examined D-amphetamine in humans using phMRI at two magnet field strengths where changes in regional cerebral blood flow (rCBF) were found in regions associated with the nigrostriatal circuit and mesolimbic and mesocortical dopaminergic pathways (Rose et al. 2006). To our knowledge, no previous imaging studies have compared the effects of the amphetamine isomers on the phMRI BOLD response in the brain of rodents or primates. This study therefore compares the phMRI BOLD response in rat brain regions after acute administration of either D-amphetamine sulphate or L-amphetamine sulphate. In vitro, behavioural and microdialysis data were used to select isomer doses with equal potency on dopaminergic systems. Thus, where the isomers produced distinct BOLD responses within an individual brain area, this is likely to reflect differential effects of the L- and D- isomers on non-dopaminergic neurotransmitter systems, such as the noradrenergic system.

Materials and methods

Animals

Male Sprague–Dawley rats (Charles River, UK) were maintained on a 12:12 h normal light/dark schedule, and all procedures were carried out in accordance with the local ethical committee, UK Animals (Scientific Procedures) Act 1986 and the Principles of Laboratory Animal Care (http://

www.nap.edu/readingroom/books/labrats.) Food and water were provided ad libitum, and room temperature (22±2°C) and humidity (40–60%) were regulated.

Drugs and dose rationale

D-Amphetamine sulphate and L-amphetamine sulphate (Boehringer-Ingelheim Chemicals, USA) were dissolved in 0.9% sterile saline made fresh on each day. Saline was used as the vehicle control and drug injected in a volume of 1 ml/kg via the intraperitoneal route. Doses of 2 mg/kg for D-amphetamine and 4 mg/kg for L-amphetamine both given i.p. were selected, as the D-isomer is reported to be twice as potent as the L-isomer on dopamine and equipotent/less potent on noradrenaline release from both in vitro (see "Introduction"), microdialysis (Kuczenski et al. 1995) and behavioural data (Dr. Terje Sagvolden, personal communication, University of Oslo, Norway).

Animal preparation

Rats (200–250 g, $n=27$) were anaesthetised with isoflurane (3% induction and then reduced to 1.75–2.0% for maintenance of anaesthesia during surgery and scanning) given in a mixture of nitrous oxide (0.4–0.6 l/min) and oxygen (0.2–0.3 l/min), varying the flow rates as required. To monitor physiological conditions, the right femoral artery was cannulated and blood pressure monitored using a transducer (AD Instruments-model: MLT0380/D). The left femoral artery was cannulated to enable blood–gas measurements to be made using an ABL 700 blood gas analyzer (Radiometer Copenhagen), and a saline infusion line was placed into the right femoral vein to maintain body fluid homeostasis. To facilitate administration of saline/drug during scanning, an intraperitoneal cannula line was inserted into the rat abdomen and anchored to the rat with cyanoacrylate. The anaesthetised rat was then transferred to an 'in-house made' cradle, designed to fit inside the probe of the Bruker 2.35 T Biospec Avance MR system (Bruker Karlsruhe, Germany). Body temperature was monitored using a rectal probe and maintained at 37±1°C via a feedback-controlled heated waterbed fitted into the cradle.

Anaesthetic consideration

The requirement for anaesthesia in rodent MRI studies is always a potential conflicting factor. Isoflurane, administered in a mixture of oxygen and nitrous oxide, was used because it produces stable anaesthesia over a prolonged period of time with minimal physiological complications. Isoflurane's effects on excitatory neurotransmission is unclear, as neuronal coupling is reported to be reduced compared to that in conscious animals, but is still detectable with 2% isoflurane

(Sicard et al. 2003). Furthermore, it has been possible to image oxygen consumption and BOLD after forepaw stimulation in isoflurane-anaesthetised rats (Liu et al. 2004; Sicard and Duong 2005). Inhalation anaesthetics have been used by several other laboratories to evaluate BOLD changes after pharmacological stimulation in rats (Cash et al. 2002; Dixon et al. 2005; Jones et al. 2005). Furthermore, any BOLD effects, which occur in amphetamine but not saline-treated rats, cannot be attributed to effects of the anaesthetic. Whilst some groups have used awake rats in short duration MRI studies (Febo et al. 2005; Skoubis et al. 2006), the current requirement of a scan for 3–4 h accompanied by administration of a stimulant drug is unlikely to be a suitable protocol for use in unanaesthetised rats.

MR methods

Radiofrequency (RF) pulses were transmitted using a 72 mm (internal) diameter birdcage coil. An electronically decoupled receive-only coil (4 cm long, 4 cm wide and 1.5 cm high) was placed on the dorsal surface of the rat's head. Magnetic resonance (MR) images were acquired using the rapid acquisition relaxation-enhanced (RARE) sequence (Hennig et al. 1986) with a field view of 50 mm. An initial anatomical volume data set (flip angle 90°, TE 62.7 ms, TR 5,112.5 ms, matrix dimensions 256×256, slice width 1 mm, slice orientation coronal, number of slices 30 with an echo train length of eight echoes, echo spacing of 15 ms and refocusing of 180°) was acquired to confirm optimal positioning of the rat within the magnet bore. This volume set was also used as an anatomical reference for subsequent functional images. The imaging parameters used for the anatomical scan yielded an in-plane spatial resolution of 0.2×0.2×1.0 mm. Anatomical and functional data were stored using Paravision 1.4 software (1996, Bruker Medizintechnik GmbH) operating on an XWIN-NMR silicon graphics console.

Scanning protocol

The current experimental design used separate saline-treated rats rather than sequential saline and drug treatment to determine the effect of the vehicle and enable statistical comparison with the effect of each amphetamine isomer in all brain regions examined. In each rat, a repetitive phMRI scanning protocol was used to study any saline- or amphet-amine-isomer-evoked changes in brain signal intensity, as measured with the T2-weighted BOLD contrast method in isoflurane-anaesthetised rats. BOLD effects were measured at 4-min 40-s intervals, over a 90-min period, at all brain levels both for basal effects (before i.p. administration) and saline/ drug effects (post i.p. administration). To determine basal activity, 12 volume data sets were acquired and recorded in the absence of drug. Vehicle (saline, i.p.) or drug (D- or L-

amphetamine) was then administered and functional imaging continued for a further 18 volume data sets. Consecutive single slice functional imaging over the whole rat brain (prefrontal cortex to brain stem) was performed to investigate the changes induced by saline/amphetamine isomer action. RARE images were recorded as continuous scanning data sets (flip angle 90°, TE 62.7 ms, TR 4356 ms, matrix dimensions 64×64, giving an unfiltered in-plane resolution of 0.8×0.8×1.0 mm) with a slice width of 1 mm, slice orientation coronal, number of slices 30 and a scan time of approximately 5 min. The length of continuous scanning can make studies prone to instrumental drift. In the present study, basal responses were stable, and we did not observe drift in the BOLD signal intensity (see "Results").

Anatomical template construction

To map regions of functional activity produced by the amphetamine isomers, a high-resolution anatomical template (256×256 matrix dimensions) was constructed on which to overlay functional maps. This was achieved by recording a volume data set of 3D coronal slices covering the rat brain for each animal. The volume data sets from each rat ($n=9$ for saline and $n=9$ for each amphetamine isomer) were then registered to an anatomical data set of Sprague–Dawley rats using the normalisation function of SPM99 (Friston et al. 1995a, b). Normalisation puts each anatomical volume data set in exactly the same space so that all grouped rat brain structures overlay exactly with one another. These data sets were then averaged (using in-house software) to give one anatomical volume data set, which served as the anatomical template. This template is used to identify brain regions showing functional activity in response to saline/drug intervention.

Functional template construction

An phMRI template is also needed because functional data collected during experimentation has different matrix dimensions (64×64). The activation data from the various rats are co-registered to produce maps of functional activity representing mean group data. The phMRI template was constructed from volume data sets (3D slices, matrix size 64×64) collected immediately after the corresponding anatomical volume data set from each rat. SPM99 was used again to co-register these volume data sets using the normalisation function to bring them into the same space. These functional data sets were then averaged using in-house software to the 3D functional template. Motion correction was achieved by re-registering each volume within the functional data sets to the middle volume acquired in each study. To eliminate movement artefacts, data were rejected if movement exceeded one third of a voxel (0.23 mm). The data sets were then

spatially filtered using a Gaussian kernel (FWHM 1.2 mm) giving a final in-plane spatial resolution of 1.2×1.2 mm. No temporal filter was applied.

BOLD determination

Data were analysed using statistical parametric maps, constructed using the general linear model formulation of SPM99, after normalisation for global effect by proportional scaling, with the haemodynamic response modelled as a boxcar function (Friston et al. 1995a, b). The experimental design consisted of an initial period of baseline recordings followed by recording of functional activity induced by saline or amphetamine isomer. The 'off' period of the boxcar function corresponds to the basal portion of collection, the duration being 60 min and the 'on' period of the boxcar model corresponds to the period of potential activity, 90 min (saline or amphetamine isomer). To determine areas of the brain demonstrating significant BOLD activity after saline or drug, two levels of analysis were performed. The first level (fixed-effect) analysis was performed on the data from individual animals and the group data. Fixed-effect analysis allows inferences to be made at the sample level and was used to determine normalised voxel signal intensities from the average co-registered subjects for later construction of time course analysis. Fixed-effect models of analysis are more sensitive than equivalent random-effect analysis because the method used draws on the large degrees of freedom and intra-subject variance. To make inferences about the group data, the fixed-effect data was subsequently processed using second level or random-effect analysis. Random-effect analysis estimates of significance are based on the mean inter-subject variance in the random-effects model. The probability threshold for random-effect analysis was $p<0.001$. Each SPM map generated had a digital rat brain atlas overlaid onto it so that each brain region could be determined. Once this overlay was deemed to be accurate (by identification of brain landmarks), using the crossbar function in SPM99, clusters of activated voxels within these identifiable brain regions (by means of the digital atlas) were identified. The bregma coordinates were obtained using anatomical markers from the acquired images and matching these markers with the anatomical digital rat atlas. Due to the histological way in which the digital rat atlas was originally drawn from, it was occasionally problematic to exactly correlate it with the acquired images where the brain is intact and, therefore, subject to CSF pressure and skull encasement. Positional accuracy of the localisation is ±1.20 mm.

Time course statistical analysis

The time course of saline or drug effects on normalised voxel signal intensities was generated from the most significant voxel within a cluster shown to be active from each co-registered subject. The mean intensity was then plotted against time. Anatomical localisation of the significant active clusters was made with reference to the anatomical template and was carried out using a crossbar facility provided in the SPM analysis package. Two-way analysis of variance (ANOVA) was used to determine if there was a main effect of drug or time and if there was any interaction. Averaged percentage change in signal intensity data after amphetamine isomer treatment were compared to averaged baseline signal intensity data using a paired Student's t test to determine if there was a significant effect after D- or L-amphetamine injection. Significance between each amphetamine isomer compared to saline at each time point was analysed by unpaired t test (between animals) and indicated by star symbols on the time courses for each level of significance. The standard error mean of normalised voxel intensities across subjects for each group was also determined for each time point.

Physiological measurements

To separate any blood pressure effects of amphetamine which could indirectly affect brain functional activity (Kalisch et al. 2001, 2005; Tuor et al. 2002), blood pressure values and respiratory rates were monitored throughout the experiment. PowerLab hardware and Chart 5.0 software was used to record changes in blood pressure (AD Instruments). Blood pressure was recorded per second, and an average was taken over 5-min time bins. Respiratory rate of each rat was also monitored in 5-min time bins. Statistical differences between saline and drug effects on blood pressure and respiratory rate was calculated using two-way ANOVA by Tukey's multiple comparison tests. Two samples of blood (55 μl) were taken during each experiment (at the beginning and at the end) for blood gas measurements.

Results

Effects of saline on positive and negative BOLD

Both fixed- (not shown) and random-effect analyses determined that compared to the pre-injection (basal) BOLD signal intensity saline evoked relatively few significant functional positive or negative changes in any rat brain region examined. The largest effects on BOLD responses were produced by saline in regions of the colliculi (−8.72 to −8.30 mm from bregma). Saline BOLD response changes were not as great as the magnitude of signal intensity changes seen with the amphetamine isomers. The changes on the BOLD response produced by saline were between −0.5 and 0.5% change in signal

intensity. Furthermore, the changes in signal intensity were not sustained for as long a duration of time as the drug induced changes, and random-effect statistical analysis shows that the changes occurred in areas of much smaller sizes than with the amphetamine isomers.

Effects of D-amphetamine sulphate on BOLD

Positive BOLD

Random-effect analysis showed that D-amphetamine produced positive BOLD changes in several discrete regions (Table 1). Some of the largest changes included the nucleus accumbens and septal nuclei (lateral and paralambdoid; Fig. 1), anterior pretectal nucleus, areas of the entorhinal cortex and retrospleniar agranular cortex (Fig. 2). All time courses showed that the BOLD signal intensity of D-amphetamine-treated rats was significantly increased compared to that in the pretreatment sample ($P<0.0001$, analysed by paired t test). Two-way ANOVA showed a significant drug versus time interaction [$F_{(1,29)}=1.79$, $P=0.008$ for lateral septal nucleus left; $F_{(1,29)}=2.20$, $P<0.0001$ for paralambdoid septal nucleus left; $F_{(1,29)}=3.81$, $P<0.0001$ for medial preoptic area; $F_{(1,29)}=2.05$, $P<0.0001$ for anterior pretectal nucleus left; $F_{(1,29)}=3.92$, $P<0.0001$ for dorsal lateral geniculate nucleus left, $F_{(1,29)}=11.28$, $P<0.0001$ for retrosplenial agranular cortex left and $F_{(1,29)}=2.79$, $P<0.0001$ for lateral dissecans

of entorhinal cortex left]. For all brain regions, there was a main effect of drug ($P<0.0001$) and time ($P<0.0001$), except for lamina dissecans of entorhinal cortex, paralambdoid and lateral septal nucleus which were only significant for drug. The time taken to reach the maximal change in signal intensity ranged from 15 to 85 min after injection of D-amphetamine.

Negative BOLD

Random-effect analysis shows that D-amphetamine sulphate produces discrete negative BOLD changes (Table 2) in caudate putamen, lateral globus pallidus, specific areas of the hippocampus (Fig. 3) and in some posterior brain regions such as the Botzinger complex and paraflocculus (data not shown). Time course analysis shows that compared to pretreatment, the BOLD signal intensity of D-amphetamine-treated rats was significantly decreased ($P<0.0001$, analysed by paired t test) in Fig. 3 such that there was a significant drug versus time interaction by two-way ANOVA [$F_{(1,29)}=2.96$, $P<0.0001$ for caudate putamen right; $F_{(1,29)}=1.807$, $P=0.0068$ for field CA3 of hippocampus; $F_{(1,29)}=6.71$, $P<0.0001$ for field CA1 of hippocampus right; $F_{(1,29)}=9.91$, $P<0.0001$ for subiculum right, $F_{(1,29)}=5.03$, $P<0.0001$ for Botzinger complex/reticular nucleus and $F_{(1,29)}=5.00$, $P<0.0001$ for paraflocculus. There were main effects of both drug ($P<0.0001$) and of time for all regions ($P<0.0001$),

Table 1 CNS areas showing significant ($p<0.001$) positive BOLD effects as analysed by random-effect analysis together with a T value >5 after injection of D-amphetamine (2 mg/kg i.p., $n=9$)

Brain regions (to be confirmed)	Bregma (mm)	Position	T value	Maximum change (% SEM)	Time to maximal change (min)
Nucleus accumbens	+2.70	Left	5.07	1.97±0.23	20
Lateral septal nucleus	+0.70	Left	5.97	0.71±0.18	50
Paralambdoid septal nucleus	+0.20	Left	5.84	0.69±0.16	40
Anterior pretectal nucleus	−4.52	Left	6.23	0.99±0.13	40
Dorsal lateral geniculate nucleus	−4.52	Left	6.22	1.49±0.11	40
Field CA1 of hippocampus	−5.20	Right	5.88	0.89±0.07	15
Forceps major of corpus callosum	−5.60	Left	5.54	0.65±0.11	40
Retrospleniar agranular cortex	−6.04	Left	5.12	1.88±0.12	50
	−6.04	Right	5.17	0.93±0.08	15
Medial entorhinal cortex	−7.64	Left	9.14	0.88±0.14	50
Lamina dissecans of entorhinal cortex	−8.00	Left	8.32	0.89±0.18	45
Deep mesencaphilic nucleus	−8.00	Right	6.32	0.81±0.12	45
Medial entorhinal cortex	−8.30	Left	6.55	0.72±0.18	60
Central nucleus of inferior colliculus or lateral periaqueductal gray	−8.72	Right	5.51	0.70±0.07	15

Whole brain BOLD effects were obtained using 17 RARE volume data sets over a 90-min period after drug injection. The maximal percentage change in mean signal intensity from basal signal intensity after drug administration and the time at which this occurred is also indicated for each region listed. Areas in the ventral pallidum, medial preoptic area, lateral posterior thalamic nucleus, gray layer of the superior colliculus and rubrospinal tract also showed significant positive BOLD responses, but had T values <5 and have been omitted from the table to focus further on key regions of change.
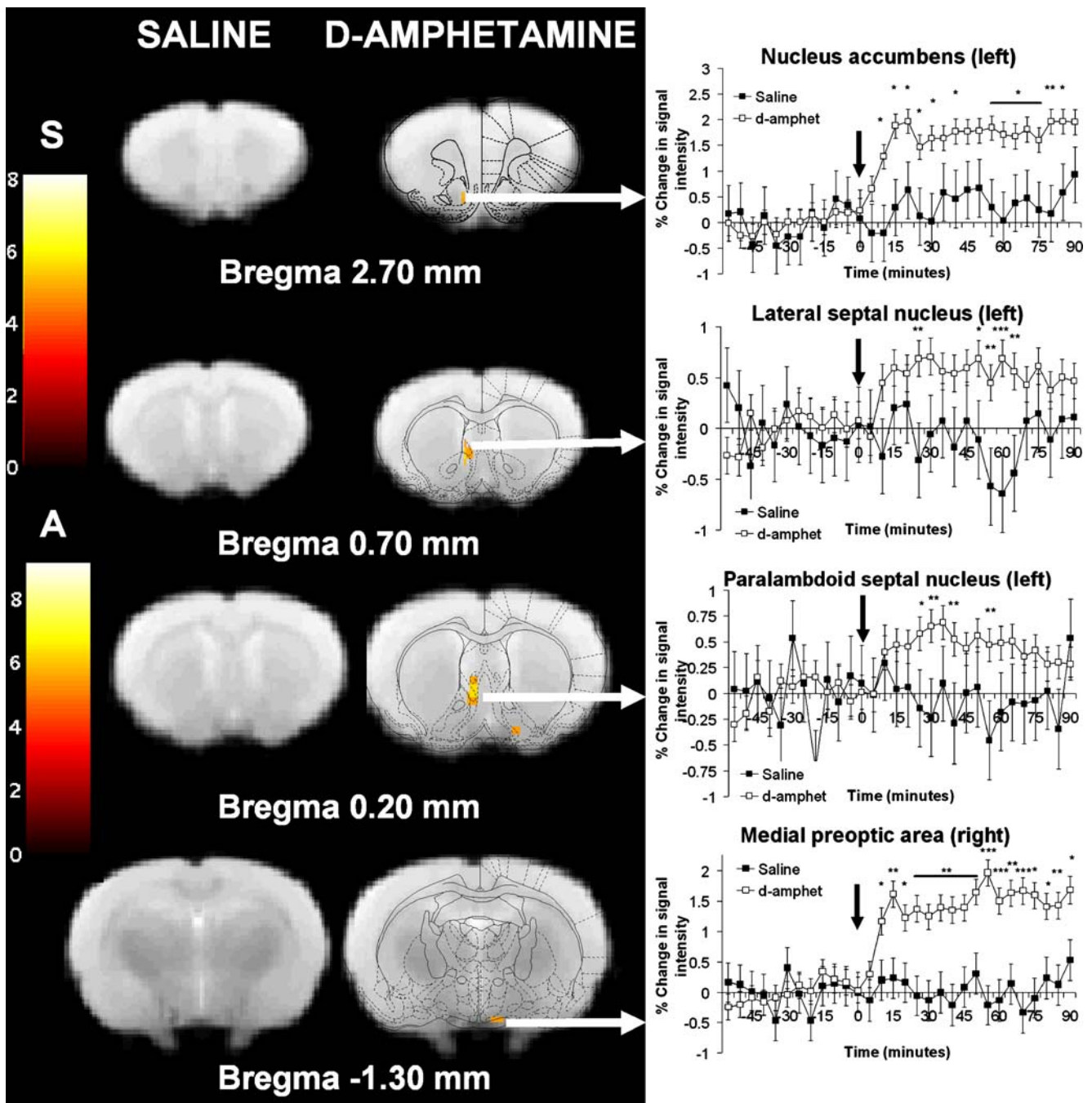
Fig. 1 Examples of positive BOLD responses in saline (*far left*) compared to respective D-amphetamine-treated rats (*left*) between 2.70 and −1.30 mm from bregma. Each image is a statistical map of the treatment used (random-effect analysis: all have *P*<0.001). Time courses (*right*) of signal intensity were obtained from fixed-effect data using coordinates for the most significant random-effect voxel from the most active cluster of voxels from either saline or D-amphetamine group data. Saline (*line with closed squares*, *n*=9) and D-amphetamine (*line with open squares*, *n*=9) are shown before and after the point of injection (denoted by *arrow*). Levels of significant differences between saline and D-amphetamine at each time point were analysed by unpaired *t* test; **P*<0.05, ***P*<0.01, ****P*<0.001. *Scale bar* shows the intensity of the BOLD effect (random-effect *T* values). *S* Saline, *A* amphetamine

except for caudate putamen (left and right which had a *P*<0.05) and lateral globus pallidus, field CA3 of hippocampus and paraflocculus which were not significant for time. The time taken to reach the maximal change in signal intensity ranged from 15 to 90 min after injection of D-amphetamine.

Effects of L-amphetamine sulphate on BOLD

Positive BOLD

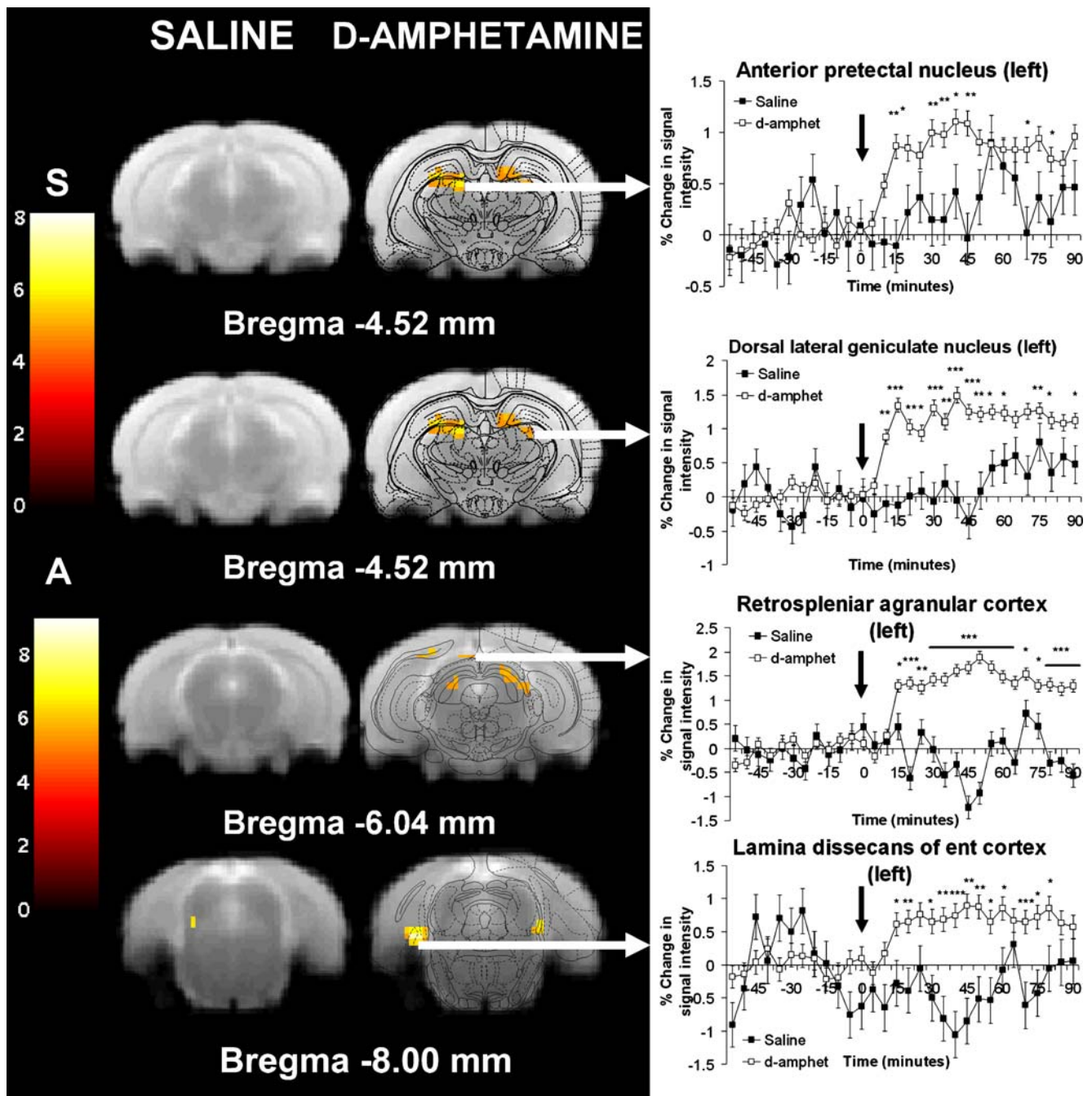Random-effect analysis showed that L-amphetamine produced positive BOLD changes which spanned from anterior

Fig. 2 Examples of positive BOLD responses in saline (*far left*) compared to D-amphetamine (*left*) treatment between −4.52 and −8.00 mm from bregma. Each image is a statistical map of the treatment used (random-effect analysis: all have *P*<0.001). Time courses (*right*) of signal intensity were obtained from fixed-effect data using coordinates for the most significant random-effect voxel from the most active cluster of voxels from either saline or D-amphetamine group data. Saline (*line with closed squares*, *n*=9) and D-amphetamine (*line with open squares*, *n*=9) are shown before and after the point of injection (denoted by *arrow*). Levels of significant differences between saline and D-amphetamine at each time point were analysed by unpaired *t* test; *P*<0.05, **P*<0.01, ***P*<0.001. *Scale bar* shows the intensity of the BOLD effect (random-effect *T* values). *S* Saline, *A* amphetamine

to posterior brain regions (Table 3 and Figs. 4, 5 and 6). All time courses showed that the BOLD signal intensity of L-amphetamine-treated rats was significantly increased compared to pre-treatment (*P*<0.0001, analysed by paired *t* test). There was a significant drug versus time interaction by two-way ANOVA [$F_{(1,29)}$=2.48, *P*<0.0001 for olfactory

ventricle/ependyma left; $F_{(1,29)}$=4.10, *P*<0.0001 for anterior olfactory nucleus left; $F_{(1,29)}$=2.00, *P*=0.0001 for lateral orbital cortex left; $F_{(1,29)}$=2.38, *P*<0.0001 for prelimbic cortex left; $F_{(1,29)}$=5.26, *P*<0.0001 for ventral orbital cortex left; $F_{(1,29)}$=3.81, *P*<0.0001 for dorsal tenia tecta left; $F_{(1,29)}$=2.81, *P*<0.0001 for nucleus accumbens left;

Table 2 CNS areas showing significant ($p<0.001$) negative BOLD effects as analysed by random-effect analysis together with a T value >5 after D-amphetamine administration (2 mg/kg i.p., $n=9$)

Brain region (to be confirmed)	Bregma (mm)	Position	T value	Maximum change (% SEM)	Time to maximal change (min)
Corpus callosum	−1.40	Left	5.79	−0.35±0.13	50
		Right	10.16	−0.45±0.09	70
Caudate putamen	−2.12	Left	7.99	−0.97±0.09	60
Field CA3 of hippocampus	−2.12	Right	6.69	−0.75±0.06	90
Field CA1 of hippocampus	−4.80	Right	9.41	−0.74±0.22	85
Subiculum	−6.80	Right	7.35	−0.87±0.07	90
Botzinger complex /reticular nucleus	−11.80	Left	7.21	−2.42±0.22	35
Paraflocculus	−11.80	Right	5.59	−1.92±0.36	65

Whole brain BOLD effects were obtained using 17 RARE volume data sets over a 90-min period after drug injection. The maximal percentage change in mean signal intensity from basal signal intensity after drug administration and the time at which this occurred is also indicated for each region listed. Areas in the right caudat putamen and lateral globus pallidus also showed significant negative BOLD responses, but had T values <5 and have been omitted from the table to focus further on key regions where maximal change was observed.

$F_{(1,29)}=2.39$, $P<0.0001$ for dorsal peduncular cortex left; $F_{(1,29)}=8.77$, $P<0.0001$ for medial forebrain bundle left; $F_{(1,29)}=5.96$, $P<0.0001$ for dentate gyrus/lateral posterior thalamic nucleus left; $F_{(1,29)}=6.13$, $P<0.0001$ for field CA1 of hippocampus; $F_{(1,29)}=8.67$, $P<0.0001$ for white layer of superior colliulus left, $F_{(1,29)}=4.22$, $P<0.0001$ for external cortex of inferior colliculus left; $F_{(1,29)}=3.55$, $P<0.0001$ for pontine reticular nucleus right and $F_{(1,29)}=8.81$, $P<0.0001$ for medial entorhinal cortex left]. There was a main effect of drug for all regions ($P<0.0001$) and time for all regions ($P<0.0001$) except for olfactory ventricle and infralimbic cortex which had lower significance ($P=0.0003$ and $P<0.0002$, respectively). The time taken to reach peak maximal change in signal intensity ranged from 10 to 90 min after injection of L-amphetamine as shown in Table 3, which lists all regions where significant changes with a T value >5 was achieved to further focus on those areas where the largest changes were observed.

Negative BOLD

Random-effect analysis shows that L-amphetamine produces marked, yet localised, negative BOLD changes (Table 4) in motor areas of the prefrontal cortex, amygdaloid nuclei, retrosplenial agranular cortex and gigantocellular reticular nucleus (Fig. 7). All time courses showed that the BOLD signal intensity of L-amphetamine-treated rats was significantly decreased compared to pre-treatment ($P<0.0001$, analysed by paired t test). Two-way ANOVA showed a significant drug versus time interaction [$F_{(1,29)}=30.71$, $P<0.0001$ for secondary motor cortex right; $F_{(1,29)}=29.56$, $P<0.0001$ for primary somatosensory cortex left; $F_{(1,29)}=2.65$, $P<0.0001$ for central amygdaloid nucleus left; $F_{(1,29)}=12.52$, $P<0.0001$ for retrosplenial agranular cortex left and $F_{(1,29)}=29.56$, $P<0.0001$ for gigantocellular reticular nucleus left]. There was also a main effect of drug and time for all regions ($P<0.0001$), except for gigantocellular reticular

nucleus which was significant for drug but not time. The time taken to reach peak maximal change in signal intensity ranged from 15 to 90 min after injection of L-amphetamine is shown in Table 4, again for all regions where a T value >5 was attained.

Physiological measurements

Blood pressure was unaffected by treatment with saline, D-amphetamine or L-amphetamine sulphate, but respiratory rate was significantly increased after amphetamine administration (Fig. 8). The L-isomer produced a drug versus time interaction [$F_{(1,29)}=1.65$, $P=0.03$] on respiratory rate and both isomers showed an effect of drug ($P<0.0001$) on respiratory rate. Blood gases were not significantly altered during the experiment (Fig. 8). Normal blood/gas values for each rat were determined by the ratio of pH, cbase and pCO_2 plotted on an acid base chart (Siggaard-Andersen 1971).

Discussion

Differences between isomers on the BOLD response

The BOLD effects of the L-isomer were more widespread and extended to more anterior and posterior brain regions than the D-isomer (Fig. 9). The larger effect of the L-isomer on positive BOLD in frontal regions may be a consequence of greater noradrenaline release, with the L-isomer in the prefrontal cortex shown in vitro (Holmes and Rutledge 1976) and in vivo (Saunders et al. 1994; Hertel et al. 1995) in the rat. It is unlikely that the difference is an effect on dopamine, as the isomers are reported to be equipotent on this at the doses tested (see dose rationale in "Materials methods"). Positive BOLD changes in the lateral and ventral orbital cortices, prelimbic cortex, dorsal tenia tecta, infralimbic cortex and dorsal peduncular cortex may be
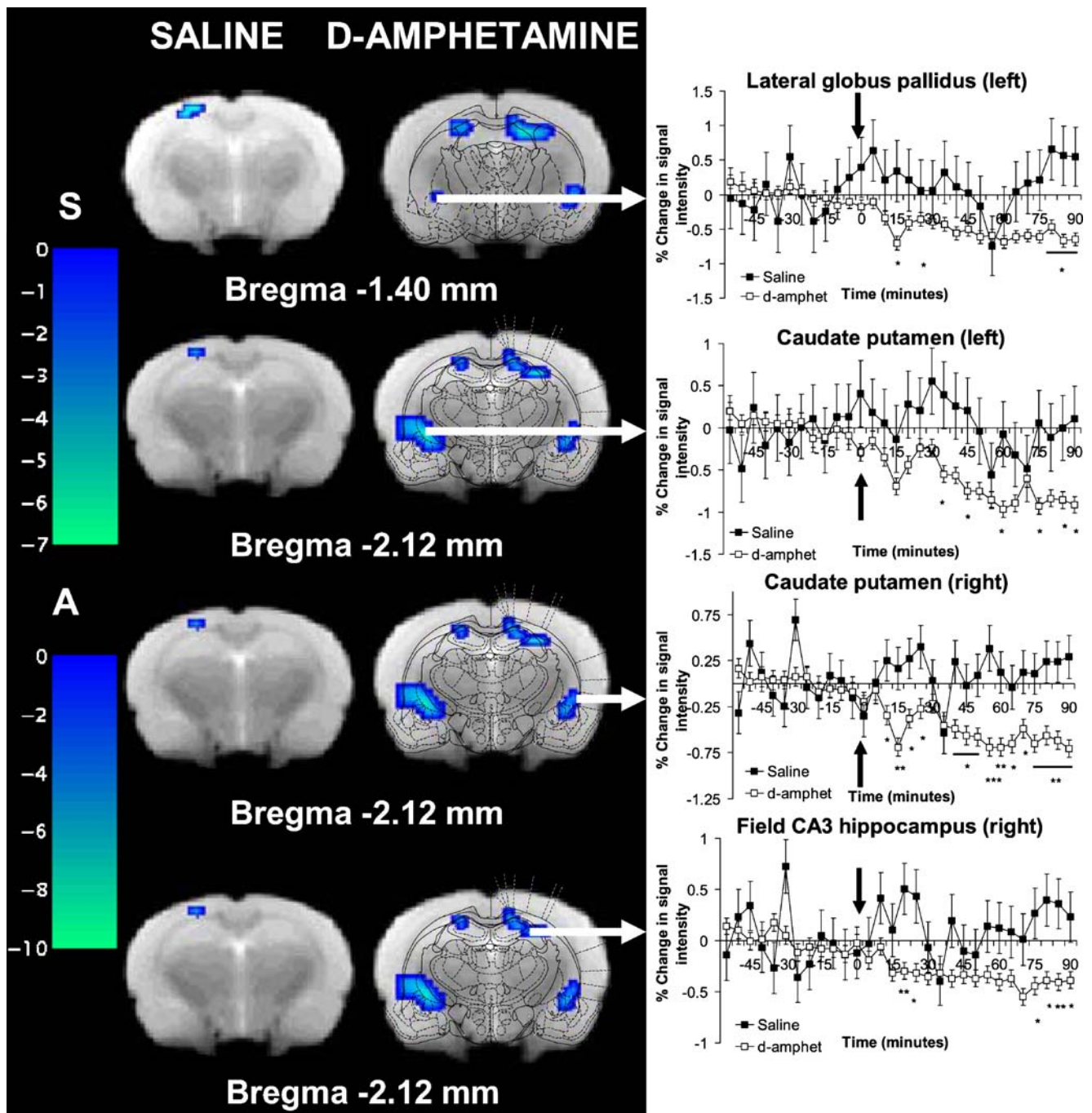
Fig. 3 Examples of negative BOLD responses in saline (*far left*) compared to respective D-amphetamine (*left*) treatment between −1.40 and −2.12 mm from bregma. Each image is a statistical map of the treatment used (random-effect analysis: all have *P*<0.001). Time courses (*right*) of signal intensity were obtained from fixed-effect data using coordinates for the most significant random-effect voxel from the most active cluster of voxels from either saline or D-amphetamine group data. Saline (*line with closed squares*, *n*=9) and D-amphetamine (*line with open squares*, *n*=9) are shown before and after the point of injection (denoted by *arrow*). Levels of significant differences between saline and D-amphetamine at each time point were analysed by unpaired *t* test; **P*<0.05, ***P*<0.01, ****P*<0.001. *Scale bar* shows the intensity of the BOLD effect (random-effect *T* values). *S* Saline, *A* amphetamine

involved in the efficacy of Aderall XR® in the treatment of ADHD. The infralimbic cortex and orbitofrontal cortex essentially constitute the ventral surface of the rat frontal cortex. Single positron emission computed tomography has shown that rCBF increases in prefrontal areas such as the

inferior orbital frontal lobe after amphetamine administration in humans (Devous et al. 2001). In the five-choice serial reaction time task, lesions of the lateral region of the orbitofrontal cortex induces 'compulsive' preservative responses, and lesions of the infralimbic cortex cause

Table 3 CNS areas showing significant ($p<0.001$) positive BOLD effects as analysed by random-effect analysis together with a T value >5 after injection of L-amphetamine (4 mg/kg i.p., $n=9$)

Brain regions (to be confirmed)	Bregma (mm)	Position	T value	Maximum change (% SEM)	Time to maximal change (min)
Olfactory ventricle/ependyma	+6.20	Left	7.35	2.84±0.30	30
Granular cell layer of olfactory bulb	+5.70	Left	7.70	2.76±0.20	30
Anterior olfactory nucleus	+5.20	Left	7.89	3.28±0.26	30
Ventral orbital cortex	+5.20	Left	5.77	3.69±0.34	20
Lateral orbital cortex	+4.70	Left	6.12	1.95±0.19	85
Prelimbic cortex	+3.70	Left	5.43	1.61±0.23	45
Dorsal tenia tecta	+3.70	Left	5.69	2.44±0.17	55
Nucleus accumbens	+2.20	Left	11.78	1.52±0.14	90
Caudate putamen	+2.20	Left	5.92	1.06±0.14	45
Dorsal peduncular cortex	+2.20	Left	5.29	1.66±0.13	45
Anterior commissure	−0.26	Left	5.64	1.11±0.07	10
Medial forebrain bundle	−2.12	Left	8.72	2.61±0.23	15
Dentate gyrus/lateral habenular nucleus	−3.60	Right	5.55	1.66±0.09	90
Anterior pretectal nucleus	−4.80	Left	5.76	1.49±0.06	80
Field CA1 of hippocampus	−6.04	Right	6.28	1.33±0.08	70
White layer of superior colliculus	−6.80	Left	6.81	1.62±0.09	80
Brachium of inferior colliculus	−7.30	Left	5.62	1.67±0.09	25
External cortex of the inferior colliulus	−8.72	Left	8.05	1.23±0.06	80
	−9.30	Right	8.05	1.47±0.21	75
Pontine reticular nucleus	−8.30	Left	5.13	1.64±0.14	10
		Right	5.03	1.70±0.15	25
Juxta/restiform body	−11.30	Left	5.33	1.10±0.17	55

Whole brain BOLD effects were obtained using 17 RARE volume data sets over a 90-min period after drug injection. The maximal percentage change in mean signal intensity from basal signal intensity after drug administration and the time at which this occurred is also indicated for each region listed. Areas in the infralimbic cortex, dentate gyrus, thalamic nucleus, gray layer of the superior colliculus, medial longitudinal fasciculus, rubrospinal tract, median raphe nucleus, medial entorhinal cortex and dorsal paragigantocellular nucleus also showed significant positive BOLD responses, but had T values <5 and have been omitted from the table to focus further on key regions where maximal drug-induced change was observed.

inappropriate 'impulsive' premature responses before the onset of stimulus (Chudasama et al. 2003). Furthermore, damage to orbitofrontal cortex regions are closely associated with disorders of 'acquired sociopathy' (Bechara et al. 1998, 1999; Rogers et al. 1999; Blair 2004), including ADHD (Casey et al. 1997). rCBF is decreased in the ventral part of middle prefrontal cortex and orbitofrontal cortex in the right hemisphere of untreated children with ADHD (Lee et al. 2005). Imaging studies have suggested a relationship between ventral frontal/orbitofrontal regions and problems with behavioural control or impulsivity in drug addiction (Volkow et al. 2001) or cocaine craving (Bonson et al. 2002). Positive BOLD changes in the prelimbic cortex may correlate with executive functions requiring attentional shift and behavioural flexibility and in working memory (Kesner 1989; Birrell and Brown 2000, De Bruin et al. 2000; Jodo et al. 2000; Mulder et al. 2000). Microdialysis studies in rats have shown that amphetamine increases extracellular noradrenaline, dopamine, glutamate and acetylcholine levels in prefrontal areas (Kankaanpaa et al. 1998; Hedou et al. 2000). Therefore, although the BOLD effects are probably attributable to effects on catecholamine systems, it

is also possible that changes produced by the L-isomer may involve release of other neurotransmitters. However, the BOLD effects could also be the indirect result of L-amphetamine's actions on other brain regions which connect with parts of the prefrontal cortex (such as the CA1 or thalamic nuclei projections). It is notable that the time to reach maximal BOLD signal intensity ranged from 10–90 min post-administration in individual regions which could, in part, reflect differences between such direct and indirect actions, but it is difficult to address the relevance of this with the current protocol.

The current study compared the effects of the amphetamine isomers by random effect analysis at a single, albeit rigorous, significance level which could cause an apparently distinct regional pattern of change due to dose differences rather than divergent pharmacological actions of the isomers. However, the pattern of BOLD changes observed at lower levels of significance introduces new areas and is still isomer-specific. It is also noteworthy that the effect of the two isomers on BOLD is opposite in some regions. The D-isomer produced positive BOLD changes in the retrosplenial agranular cortex compared to negative changes evoked by the L-isomer. In
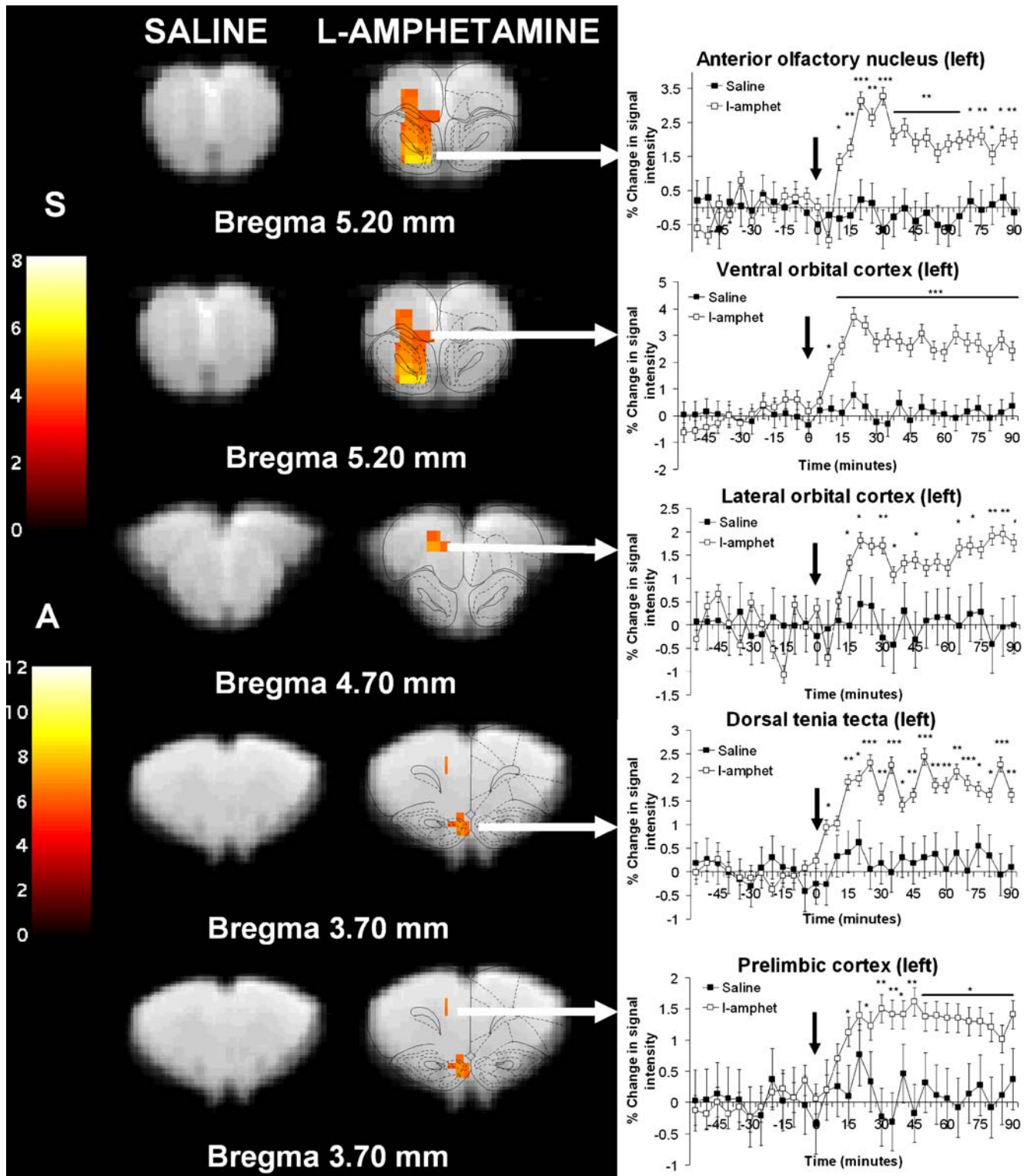
Fig. 4 Examples of positive BOLD responses in saline (*far left*) and ʟ-amphetamine-treated rats (*left*) between 5.20 and 3.70 mm from bregma. Each image is a statistical map of the treatment used (random-effect analysis: all have $P<0.001$). Time courses (*right*) of signal intensity were obtained from fixed-effect data using coordinates for the most significant random-effect voxel from the most active cluster of voxels from either saline or ʟ-amphetamine group data.

Saline (*line with closed squares*, $n=9$) and ʟ-amphetamine (*line with open squares*, $n=9$) are shown before and after the point of injection (denoted by *arrow*). Levels of significant differences between saline and ʟ-amphetamine at each time point were analysed by unpaired t test; *$P<0.05$, **$P<0.01$, ***$P<0.001$. *Scale bar* shows the intensity of the BOLD effect (random-effect T values). *S* Saline, *A* amphetamine
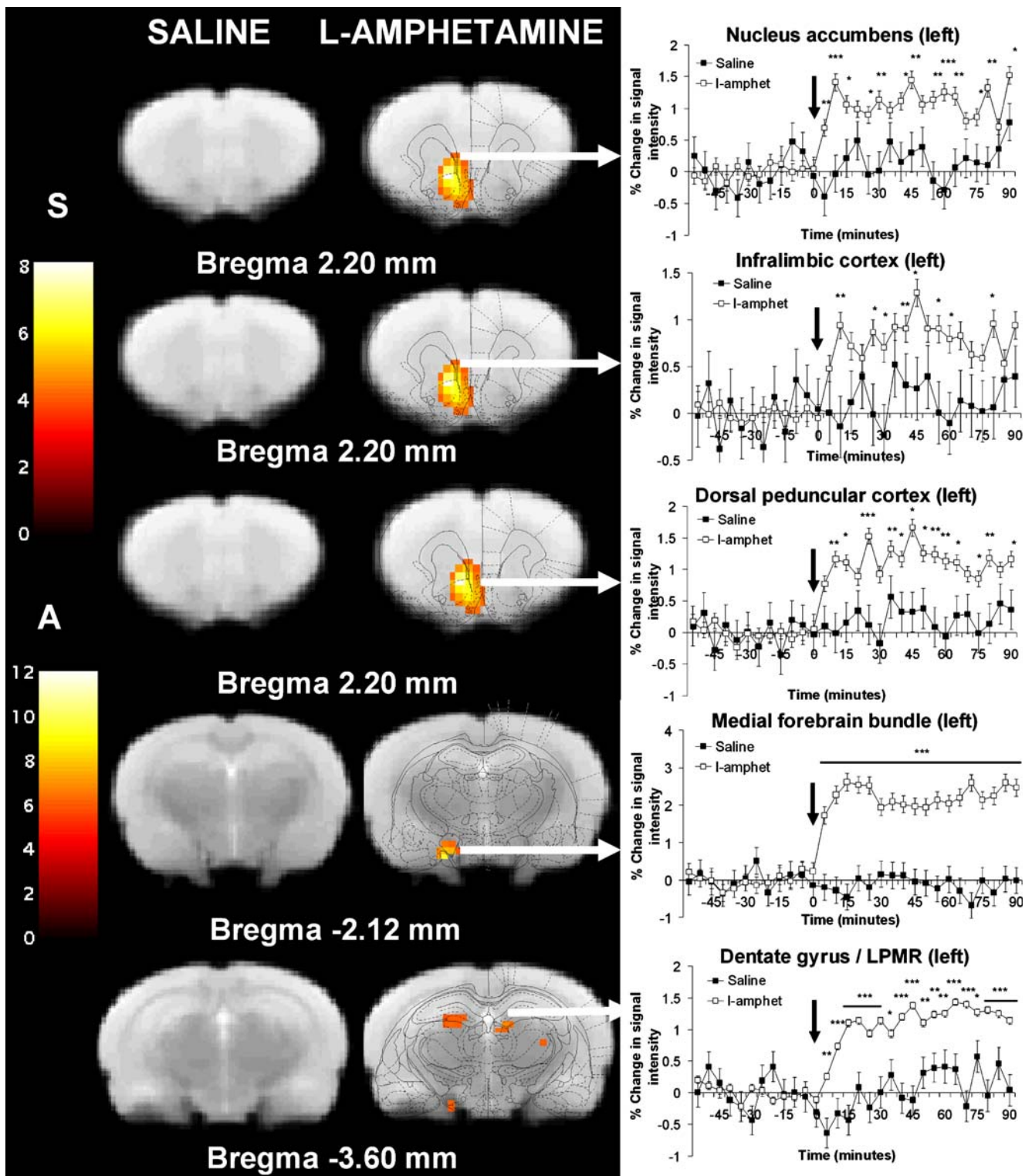
Fig. 5 Examples of positive BOLD responses in saline (*far left*) compared to ʟ-amphetamine-treated rats (*left*) between 2.20 and −3.60 mm from bregma. Each image is a statistical map of the treatment used (random-effect analysis: all have *P*<0.001). Time courses (*right*) of signal intensity were obtained from fixed-effect data using coordinates for the most significant random-effect voxel from the most active cluster of voxels from either saline or ʟ-amphetamine group data. Saline (*line with closed squares*, *n*=9) and ʟ-amphetamine (*line with open squares*, *n*=9) are shown before and after the point of injection (denoted by *arrow*). Levels of significant differences between saline and ʟ-amphetamine at each time point were analysed by unpaired *t* test; **P*<0.05, ***P*<0.01, ****P*<0.001. *Scale bar* shows the intensity of the BOLD effect (random-effect *T* values). *S* Saline, *A* amphetamine
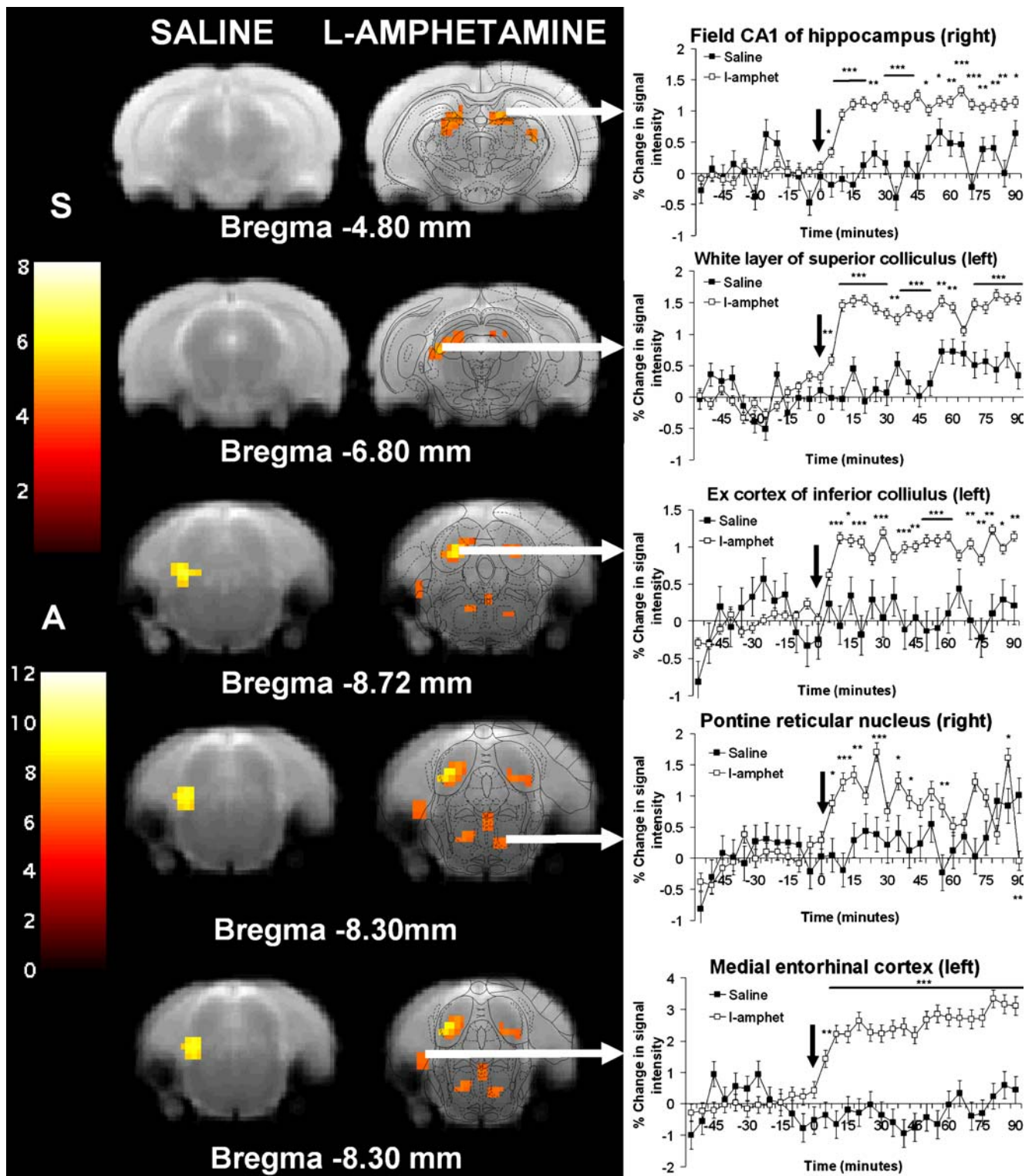
Fig. 6 Examples of positive BOLD responses in saline (*far left*) compared to respective L-amphetamine-treated rats (*left*) between −4.80 and −8.30 mm from bregma. Each image is a statistical map of the treatment used (random-effect analysis: all have $P<0.001$). Time courses (*right*) of signal intensity were obtained from fixed-effect data using coordinates for the most significant random-effect voxel from the most active cluster of voxels from either saline or L-amphetamine group data. Saline (*line with closed squares*, $n=9$) and L-amphetamine (*line with open squares*, $n=9$) are shown before and after the point of injection (denoted by *arrow*). Levels of significant differences between saline and L-amphetamine at each time point were analysed by unpaired t test; *$P<0.05$, **$P<0.01$, ***$P<0.001$. *Scale bar* shows the intensity of the BOLD effect (random-effect T values). *S* Saline, *A* amphetamine

Table 4 CNS areas showing significant ($p<0.001$) negative BOLD effects as analysed by random effect analysis together with a T value >5 after L-amphetamine administration (4 mg/kg i.p., $n=9$)

Brain region (to be confirmed)	Bregma (mm)	Position	T value	Maximum change (% SEM)	Time to maximal change (min)
Primary motor cortex	+0.70	Left	6.29	−5.94±0.50	85
Secondary motor cortex	+0.48	Right	5.23	−7.43±0.45	85
Primary somatosensory cortex	−1.80	Left	7.99	−0.97±0.09	60
Secondary somatosensory cortex	−2.12	Left	5.28	−1.11±0.09	85
Field CA1/CA2 of hippocampus	−3.30	Right	6.11	−0.83±0.05	90
Retrosplenial agranular cortex	−6.72	Left	4.61	−3.74±0.42	85
Gigantocellular reticular nucleus	−11.30	Left	6.76	−1.30±0.15	65

Whole brain BOLD effects were obtained using 17 RARE volume data sets over a 90-min period after drug injection. The maximal percentage change in mean signal intensity from basal signal intensity after drug administration and the time at which this occurred is also indicated for each region listed. Areas in the central amygdaloid nucleus and posterior hypothalamic area also showed significant ($p<0.001$) negative BOLD responses, but had T values <5 and have been omitted from the table to focus further on key regions where change was maximal.

contrast, negative BOLD changes occurred with the D-isomer in the caudate putamen and CA1 region of the hippocampus, whereas positive BOLD was produced by the L-isomer in these regions. These effects suggest a brain-region-specific stereoselective action of the isomers, which may reflect their different potencies on dopamine and noradrenaline reuptake and release and make it unlikely that the divergent pattern of brain regional changes in BOLD produced by the two isomers is due to the specific level of significance examined.

The contrasting effects in the caudate putamen may result from isomeric differences in dopamine transmission in the striatum and/or on noradrenaline in the prefrontal cortex (Heikkila et al. 1975; Holmes and Rutledge 1976). The L-isomer produced discrete positive changes, and the D-isomer produced only small bilateral negative BOLD changes in the caudate putamen. The effects seen with the L-isomer are complicated further, as activity in the frontal cortex is known to exert inhibitory influence on subcortical brain regions such as the caudate putamen (Jaskiw et al. 1991a, b; Murase et al. 1993; Kolachana et al. 1995; Karreman and Moghaddam 1996; Dalley et al. 1999; Jackson et al. 2001). It is therefore likely that the brain regions activated by the L-isomer either do not exert inhibitory influence on striatal regions or its influence was not detected. This is supported by the fact that the lateral orbital portion of the frontal cortex does not project to the ventromedial region of the striatum (Phillipson and Griffiths 1985; Brog et al. 1993).

Negative BOLD changes specific to the L-isomer were produced bilaterally in the motor and somatosensory cortices. In humans, activation of the primary and secondary motor areas is associated with the planning and execution of movements, motor control (Graziano et al. 2002), learning (Sanes 2003) and skill acquisition (Scott 2000), so the large negative BOLD changes produced in these areas may reflect a disruption of these behaviours. Furthermore, the decreased rCBF seen in these regions after administration in humans (Devous et al. 2001) supports the action of amphetamine in

motor cortical areas. Amphetamine has analgesic properties (Shimm et al. 1979; Clarke and Franklin 1992) and can increase pain thresholds in humans (Webb et al. 1978). The negative BOLD responses in somatosensory areas may indicate decreased neuronal activation and could contribute to these analgesic effects. Increased BOLD responses after administration of both isomers occurred in the anterior pretectal nucleus (APTN). 2-deoxyglucose uptake (indicative of brain activity) is increased in the APTN after amphetamine injection in mice (Miyamoto et al. 2000), and APTN stimulation produces antinociception (Rees and Roberts 1987). Antinociceptive effects of the APTN are mediated in part via a relay through the deep mesencephalic nucleus (DPMe), which also showed BOLD activation after D- but not the L-amphetamine administration (Wang et al. 1992). The DPMe projects to the pontine reticular and gigantocellular nucleus, and projections to the DPMe arise from somatosensory cortices in the rat (Veazey and Severin 1980) which, the BOLD responses show, were altered by L-amphetamine.

Only the L-isomer produced positive BOLD changes in the medial forebrain bundle (MFB). Activation of the MFB probably contributes to the well-documented rewarding effects (Olds and Olds 1963), and this stimulation is probably dependent on activity of the mesocorticolimbic dopamine system (Wise 1980; Yeomans 1982). The MFB runs from the centre of the brain (the ventral tegmental area or VTA), through the lateral hypothalamus, the nucleus accumbens (a major relay system) and up to the frontal cortex. It is widely considered that not only drugs, but also social experiences, that produce pleasure act on this complex pathway. The D-isomer produced effects on 'pleasure-related' brain areas unrelated to the MFB, such as the septal nuclei and ventral pallidum. Evidence suggests activity in a subcortical network involving portions of the nucleus accumbens shell, ventral pallidum, and brainstem causes 'liking' and positive affective reactions to sweet tastes and lesions of ventral pallidum also impair normal sensory
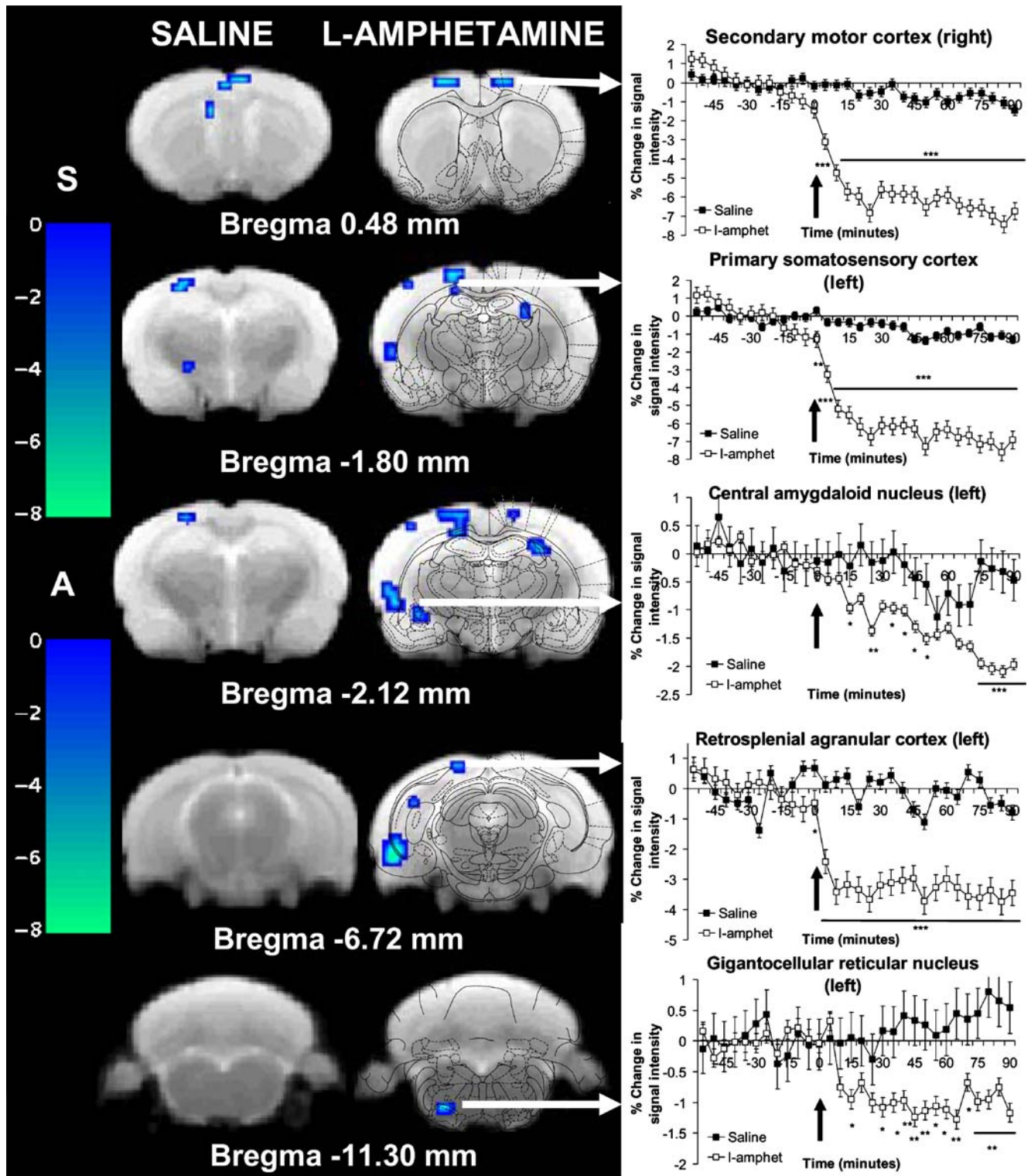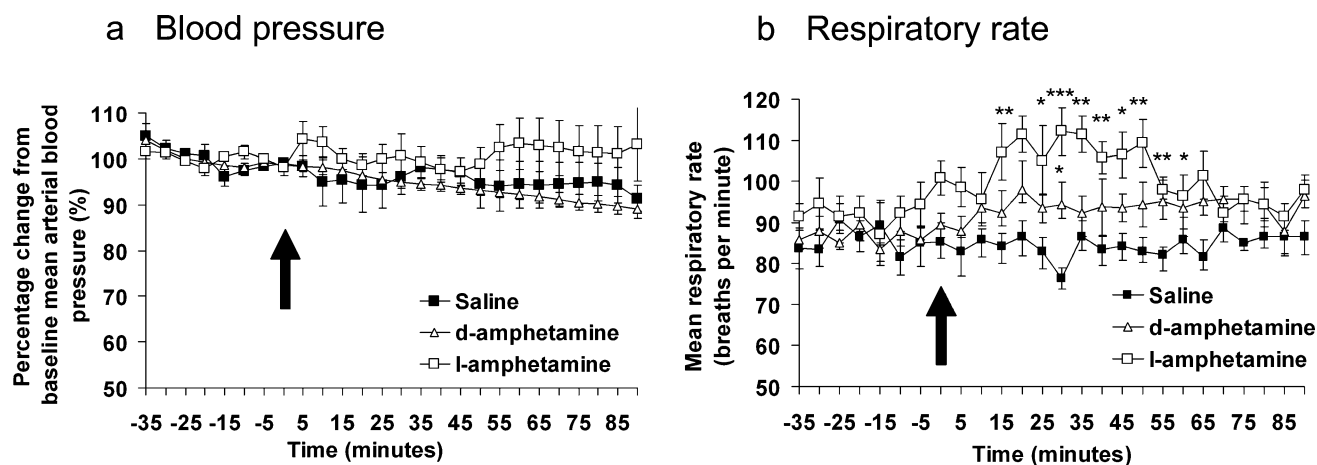
Fig. 7 Examples of negative BOLD responses in saline (*far left*) compared to respective L-amphetamine-treated rats (*left*) between 0.48 and −11.30 mm from bregma. Each image is a statistical map of the treatment used (random-effect analysis: all have *P*<0.001). Time courses (*right*) of signal intensity were obtained from fixed-effect data using coordinates for the most significant random-effect voxel from the most active cluster of voxels from either saline or L-amphetamine group data. Saline (*line with closed squares*, *n*=9) and L-amphetamine (*line with open squares*, *n*=9) are shown before and after the point of injection (denoted by *arrow*). Levels of significant differences between saline and L-amphetamine at each time point were analysed by unpaired *t* test; **P*<0.05, ***P*<0.01, ****P*<0.001. *Scale bar* shows the intensity of the BOLD effect (random-effect *T* values). *S* Saline, *A* amphetamine

a Blood pressure

b Respiratory rate

	Values at START of scanning			Values at END of scanning		
Treatment	pH	pCO$_2$ (mmHg)	cBase (Ecf) (mmol/L)	pH	pCO$_2$ (mmHg)	cBase (Ecf) (mmol/L)
Saline	7.38 (± 0.011)	46.95 (± 1.797)	3.01 (± 0.554)	7.41 (± 0.011)	41.89 (± 1.872)	1.74 (± 0.414)
D-amphetamine	7.40 (± 0.010)	43.47 (± 1.621)	2.15 (± 0.503)	7.38 (± 0.011)	45.04 (± 1.555)	1.44 (± 0.555)
L-amphetamine	7.39 (± 0.014)	45.39 (± 2.605)	2.67 (± 1.276)	7.40 (± 0.010)	45.78 (± 1.665)	2.58 (± 0.885)

Fig. 8 a Percentage change of arterial blood pressure from baseline data and **b** mean respiratory rate before and after saline, D-amphetamine (2 mg/kg, i.p.) or L-amphetamine injection (4 mg/kg, i.p.). *Arrows* indicate the point of injection. Each point represents the percentage change in arterial pressure±SEM of the group of rats or mean breaths taken per minute (*n*=6–7). Levels of significant differences between saline and either D-amphetamine or L-amphetamine were analysed by two-way ANOVA for drug versus time interaction. Differences between drug and saline at each time point was examined by one-way ANOVA followed by Tukey's multiple comparison test; *$P<0.05$, **$P<0.01$, ***$P<0.001$

pleasure (Berridge 2003). The present data therefore show that 'pleasure-areas' of the rat brain show activation, but that these areas differ for each amphetamine isomer. The positive BOLD changes produced by the L-isomer in the median raphe nucleus also indicate that it may alter 5-HT neurotransmission between the midbrain and forebrain.

Similarities of the isomers on the BOLD response

Both isomers produced positive BOLD changes in the shell and core regions of the nucleus accumbens. These regions are dissociable, the medial shell being involved in reward and the core in the locomotor stimulant effects of amphetamine (Sellings and Clarke 2003). These changes may relate to release of dopamine by amphetamine in the nucleus accumbens (Sharp et al. 1987; Carboni et al. 1989; Giorgi et al. 2005). Lesions to the nucleus accumbens result in hypoactivity and decrease the locomotor-stimulating effect of amphetamine (Parkinson et al. 1999; Sellings and Clarke

2003). So, it is unclear how activation of the nucleus accumbens by amphetamine is beneficial in the treatment of ADHD hyperactivity, but does explain how acute administration of either isomer increases locomotor activity in rats (Concannon and Schechter 1982). Both isomers produced positive BOLD changes in the colliculi. This may be a result of blood flow and baseline uptake of deoxyglucose being highest in the inferior colliculus which is the most metabolically active structure in the brain (Sokoloff 1981). However, examination of the time courses for these regions shows a clear increase in the BOLD response after amphetamine injection. The superior colliculi in particular are involved in the control of covert spatial attention, a process that focuses attention on a region of space different from the point of gaze (Goldberg and Wurtz 1972; Ignashchenkova et al. 2004). Microstimulation of the superior colliculi in monkeys focuses attention without moving the eyes (Muller et al. 2005), suggesting increased activity in the colliculi may help focus attention. Jane et al.

Fig. 9 Probability maps (*col-oured areas*) overlayed onto anatomical templates (*grey*) which show the number of animals in each amphetamine treatment group producing changes in the positive BOLD response in prefrontal regions of rat brain (1.70 to 6.70 mm from bregma) from a total group size of nine rats. Images at the *bottom* show the lateral view at two dorso-ventral coordinates (4.00 and 5.50 mm from bregma). *Scale bar* shows the number of animals which produced an effect after amphetamine isomer administration
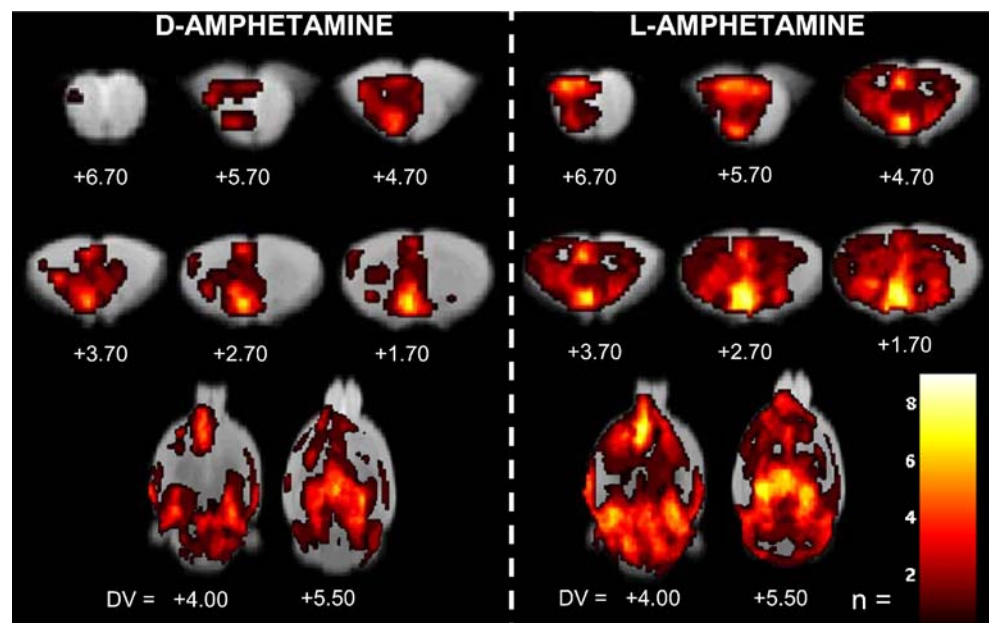
(1965) reported a loss of attention and behavioural changes in cats after severing the brachium of the inferior colliculus (its connection to the thalamus). Similarly, Sprague (1991) observed a dramatic loss of attention, affect and motivation in cats after severing the lateral lemniscal tracts between the superior olive and inferior colliculus, commenting that these behavioural changes were reminiscent of autistic children when comparing their findings with those of Kluver and Bucy (1997) who obtained similar behavioural deficits after removing the temporal lobes of monkeys. Both isomers increased BOLD responses in the medial entorhinal cortex. Lesions of this brain region in mice (Schenk et al. 1983) and rats (Kohler and Sundberg 1977) produce increased locomotor activity and exploratory behaviour. Activation of this brain region may, therefore, contribute to the regulation of hyperactivity and decrease exploratory behaviour.

Effects of the isomers on respiratory rate

Respiration can affect the phMRI time series by changing the arterial level of CO_2 (a potent vasodilator which can produce an increase in cerebral blood flow and, hence, an increase in the BOLD signal). This increase in response to hypercapnia has been studied extensively in fMRI using both the administration of CO_2 and breath-holding in humans (Bandettini and Wong 1997; Davis et al. 1998; Kastrup et al. 1999a, b; Rostrup et al. 2000). Similarly, a decrease in arterial CO_2 results in a decreased rCBF and a decreased BOLD signal, as shown by fMRI studies using hyperventilation (Posse et al. 2001). The L-isomer had an effect on respiratory rate, although blood gas parameters remained within normal physiological limits (where respiratory rate is reported to be between 70 and 120 breaths per minute for an

anaesthetised rat, Flecknell and Waynsworth 1992). Examination of the BOLD time courses shows that signal changes continue even after respiration rate returns to normal. This is a good indicator that the BOLD changes are related to the central effects of the drug and not secondary to changes in blood CO_2 as has been demonstrated with cocaine in the rat (Schmidt et al. 2006). Furthermore, if the increased respiration rate had produced BOLD responses, then these would have been negative. The majority of effects with the amphetamines were positive BOLD changes, which, if they were the consequence of change in respiration rate, would have been unexpected. Additionally, arterial blood CO_2 concentration changed less than it did in our saline-treated rats during the scanning period, which would indicate that hypo/hypercapnia had not occurred. D-Amphetamine caused a dose-dependent increase in respiratory rate in anaesthetised rats, and brain regions involved in this effect include the rostral pontine structures in the region of the medial parabrachial nuclei and the reticular respiratory oscillator (Mediavilla et al. 1979). As the L-isomer is more effective at promoting the availability of noradrenaline at postsynaptic receptors, this may enhance the inspiratory 'off-switch' within pontine structures. Noradrenergic fibres originate in the parabrachial area and project to the pons and medulla oblongata which are involved in autonomic functions such as breathing and heart rate (Sachs et al. 1973) consistent with this suggestion.

Conclusions

Whilst the amphetamine isomers exhibited similar effects on the BOLD response in several brain regions, the L-

isomer produced more widespread effects and, in particular, activation in the fronto-cortical brain regions not seen with the D-isomer. These frontal areas are convincingly linked with attention in humans (Godefroy and Rousseaux 1996; Wilkins et al. 1987; Manes et al. 2002), and prefrontal cortex lesions have been shown to impair attention in monkeys (Malmo 1942; Bartus and Levere 1977; Dias et al. 1996) and rats (Muir et al. 1996). The ability of the amphetamine isomers to produce different BOLD responses in brain areas related to cognition, pleasure, pain processing and motor control may reflect isomeric variations on brain amine systems such as dopamine and noradrenaline and suggests that the isomers have distinct actions on brain regions thought to be impaired in ADHD patients. Further studies are required to examine the role of dopamine and noradrenaline in the specific brain region BOLD effects produced by the isomers. Such studies should include the use of agents with selectivity for individual monoamine receptors to establish the pharmacological specificity of the action of the amphetamine isomers on the BOLD response.

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