CANNABINOIDS IN EXPERIMENTAL STROKE: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Abstract

Cannabinoids (CB) show promise as neuroprotectants with some agents already licensed in humans for other conditions. We systematically reviewed CBs in pre-clinical stroke to guide further experimental protocols. We selected controlled studies assessing acute administration of CBs for experiment stroke, identified through systematic searches. Data were extracted on lesion volume, outcome and quality; analysed using random effects models; results are expressed as standardised mean difference (SMD) with 95% confidence intervals [CI]. 144 experiments (34 publications) assessed CBs on infarct volume in 1473 animals. CBs reduced infarct volume in transient (SMD -1.41, [95% CI -1.71,-1.11], p<0.00001) and permanent (-1.67 [-2.08,-1.27], p<0.00001) ischaemia and in all subclasses: endocannabinoids (-1.72 [-2.62,-0.82], p=0.0002), CB_1/CB_2 ligands (-1.75 [-2.19,-1.31], p<0.00001), CB_2 ligands (-1.65 [-2.09,-1.22], p<0.00001), cannabidiol (-1.20 [-1.63,-0.77], p<0.00001), Δ^9-tetrahydrocannabinol (-1.43 [-2.01,-0.86], p<0.00001) and HU-211 (-2.90 [-4.24,-1.56], p<0.0001). Early and late neuroscores significantly improved with cannabinoid use (-1.27 [-1.58,-0.95], p<0.00001; -1.63 [-2.64,-0.62], p<0.002 respectively) and there was no effect on survival. Statistical heterogeneity and publication bias was present, median study quality was 4 (range 1-6/8). Overall, CBs significantly reduced infarct volume and improve functional outcome in experimental stroke. Further studies in aged, female and larger animals, with other co-morbidities are required.

Key words: stroke, neuroprotection, meta-analysis, cannabinoid, pre-clinical

Introduction

Components of the endocannabinoid system (ECS) are altered following ischaemic stroke. The expression of CB_1 and CB_2 receptors are upregulated in the rat brain following cerebral ischaemia,[1, 2] indicating that the ECS may play an important role in the endogenous response to stroke, though the relevance of these changes are not known. Human and animal *in vivo* data have shown increases in neurological levels of anandamide (AEA), oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), with 2-arachidonoylglycerol (2-AG) levels either unchanged or increased.[3-8] Pre-clinical stroke studies have derived neuroprotective qualities from a range of approaches to manipulating the ECS. For example, CB_2 ligands can modify the post-stroke inflammatory response, and CB_1 activation can initiate a chemical hypothermia, with both processes resulting in a decrease in stroke infarct volume. [9, 10] Activation of CB_2 receptors has only demonstrated protective effects and the role of CB_1 activation is less clear with studies demonstrating efficacy of both CB_1 agonists and antagonists.[11, 12]

Cannabinoids can be divided into three categories: endocannabinoids, phytocannabinoids and synthetic cannabinoids. AEA and 2-AG (both $CB_{1/2}$ agonists) are the best studied endocannabinoids, but other chemically similar compounds have been suggested as endocannabinoids or endocannabinoid-like compounds, including OEA, PEA, lauroylethanolamide and linoleoylethanolamide. Endocannabinoids also display activity at non-$CB_{1/2}$ receptor sites, including TRPV1, PPARα/γ, $5HT_{1A}$ and GPR55.[13] Phytocannabinoids are derived from the cannabis plant, a unique source of over 60 different compounds, with Δ^9-tetrahydrocannabinol (THC) and

cannabidiol (CBD) already in clinical use to treat spasticity in multiple sclerosis (Sativex). THC is a partial agonist for CB_1 and CB_2 receptors, whilst CBD displays low affinity for CB receptors.[14] Synthetic cannabinoid compounds have been developed, some of which exhibit high potency at CB_1 (arachidonyl-2'-chloroethylamide) or CB_2 (JWH-133, O-1966 and O-3853) receptors, activate both $CB_{1/2}$ (CP 55,940, HU-210, TAK-937 and WIN 55,212-2), or activate non-CB receptors (e.g. HU-211, a proposed NMDA antagonist).

Given the accumulating preclinical evidence for the use of cannabinoids in stroke, as well as the expansion in the use of cannabinoid-based medicines in other disorders, a systematic review of the currently available preclinical literature is warranted. Whilst it is clear there are many studies describing the benefit of administering cannabinoids for experimental stroke, a number of unanswered questions remain before the transition is made into 'bedside' testing. It is unclear as to whether the optimal time of administration and dose of the various cannabinoid classes have been established, and whether the body of evidence is reliable and consistent. The aim of this study, therefore, is to systematically review and meta-analyse the effects of exogenous cannabinoid administration on infarct volume, functional outcome and survival in animal models of ischaemic stroke.

Methods

Search Criteria

Experimental (non-human) studies in evaluating the effect of cannabinoids on focal acute stroke were searched up to December 2013 in PubMed, Medline, Embase, ScienceDirect, and Web Of Science. Search keywords included were 'stroke,' 'ischaemia,' 'cannabinoid,' 'cannabidiol,' 'delta-9-tetrahydrocannabinoid,' 'WIN 55,212-2,' '2-Arachidonoy glycerol,' 'endocannabinoids,' 'CB_1 receptors,' and 'CB_2 receptors.' References from included studies and conference proceedings were also searched. There was no protocol per se, although pre-specified exclusion criteria were used to prevent bias and studies were included if the following were met: (i) a focal ischaemic stroke model, not global; (ii) treatment was given for an acute model (within 48 hours), not chronic; (iii) only cannabinoid ligands were given; (iv) there was a control group; (v) there were measures of infarct size, functional outcome or survival; and (vi) data was from an original article, not a review article. If an article was only available as an abstract it was not included.

Data acquisition

Data on total infarct size, measured in percentage (%) or volume (mm^3) were extracted from included papers. Volumes corrected for oedema were chosen instead of uncorrected data. When all data was not available, authors were contacted for the exact numbers of animals used in each group for each

experiment. If authors were unable to provide necessary information, the lowest number of animals within the range given was used. The Grab application (version 1.5) was used to obtain values from figures given in published articles if no values were stated within the text. Similarly, information on vital status, weight (grams), Rotarod test (time spent on Rotarod expressed in seconds or percentage compared to baseline) and neurological score were collected. If published articles used multiple groups (e.g., to assess time response relationships) with one control group, then the number of animals per control group was divided into the number of comparison groups. Since different procedures were used in different experiments, the total dose of drug given throughout a complete experiment was taken instead of a single dose. When drugs were given at more than one point of time, the earliest time of administration was used.

Quality

Methodological quality was assessed using an eight-point criteria derived from STAIR [15], as used previously, [16, 17] with 1 point given to evidence of the following: presence of randomisation, monitoring temperature throughout the experiment, masked outcome measurement, assessment of outcome at days 1-3, assessment of outcome at days 7-30, assessment of outcome other than just infarct size, dose-response relationship conducted, and therapeutic time window relationship of a particular agonist conducted.

Data analysis

Data were grouped before analysis by (i) model type (permanent or transient ischaemia); (ii) species; (iii) time to treatment; (iv) total dose and (v) cannabinoid type. Data from each of these groups were analysed as forest plots using the Cochrane Review Manager software (version 5.2) and Stata (version 11), as used in previous animal meta-analyses.[17] Since heterogeneity was expected between study protocols (different species, stroke models, dose, time), random effect models were used. The results of continuous data are expressed as standardised mean difference (SMD), with 95% confidence intervals (CI), which allows data measured on different scales and in different species to be merged. The results of binary data (survival) are expressed as odds ratios (OR) with 95% CI. Studies were weighted by sample size and statistical significance was set at $p<0.05$. PRISM 6 (GraphPad) was used to compare the dose- and time-response relationship between drug classes. Infarct volume data acquired can be accessed at http://dx.doi.org/10.6084/m9.figshare.1228070.

Results

Design of the studies

The initial search for studies identified 101 relevant publications. Once the pre-specified inclusion criteria were applied, a total of 34 publications were chosen for analysis (Figure 1, Table 1). These came from 18 laboratories in 9 countries (USA, Israel, Italy, Japan, Spain, Denmark, Germany, UK and China). Studies were excluded if they examined global ischaemia, neonatal animals, did not measure infarct volume or functional outcome, did not administer a cannabinoid receptor ligand, were review articles (not original articles), where induction of injury was by methods other than ischaemia/reperfusion or if the data was unobtainable.

19 of 34 publications studied 786 rats [5, 10, 18-34] and 14 studied 673 mice;[3, 9, 11, 12, 35-44] 1 article studied rats and primates.[45] 24 articles examined greater than 1 experimental paradigm (total number of experiments 144). Transient ischaemic models were used in 21 publications, vessel occlusion time ranging between 30 minutes to 4 hours. Permanent models of ischaemia were used in 13 articles (photothrombotic n=2). Drugs were administered intravenously (n=17) or via the peritoneum (n=16), and one study used the oral route.[44] Time of administration ranged from pre-ischaemia up to 5 days post middle cerebral artery occlusion (MCAo). Median study quality was 4 (range 1-6).

Infarct volume

Overall, administration of cannabinoid receptor ligands reduced infarct volume in comparison to vehicle; standardised mean difference, SMD, -1.49, 95% confidence interval CI, -1.73 to -1.25, $p < 0.00001$ (Figure 2, Table 2). If we only include the 18 publications (75 experiments, 767 animals) reporting absolute lesion volume in the analysis, the weighted mean difference (WMD) between groups was -28.3 mm^3 (95% CI -32.4, -24.2, $p<0.00001$) in favour of cannabinoids; equivalent to a SMD of -1.27 (95% CI -1.58, -0.97, $p<0.00001$).

Infarct volume was significantly reduced in rats and mice, SMD -1.75, (95% CI -2.15 to -1.35, $p < 0.00001$) and -1.34 (95% CI -1.61 to -1.06, $p < 0.00001$) respectively. The only study involving primates revealed non-significant infarct volume reduction upon administration of TAK-937, a CB_1/CB_2 receptor ligand (SMD -0.55, 95% CI -1.62 to 0.53, $p = 0.32$).[45]

When grouped by drug class, synthetic agonists (mixed CB_1/CB_2 ligands ($p<0.00001$), CB_2 ligands ($p<0.00001$), HU-211 ($p<0.0001$)), phytocannabinoids (THC and CBD (both $p<0.00001$)) and endocannabinoids ($p=0.002$) all reduced infarct volume significantly (see Table 2). The breakdown by individual compound can be seen in Figure 2; the most profound infarct volume reduction is seen with HU-210, a synthetic CB_1/CB_2 ligand (n=8 experiments, 80 animals [10]) (SMD -3.52, 95% CI –5.34 to -1.71). Methanandamide, lauroylethanolamide and linoleoylethanolamide were all neutral in their effect, whereas AEA (n=3, 28 animals) showed borderline significant infarct volume reduction (SMD -0.78, 95% CI –1.64 to 0.08, $p=0.07$).

Individual studies of the CB_1 antagonist SR141716 had a neutral effect on lesion volume except when used at a very high dose (20 mg/kg [11]) leading to a significant reduction in lesion size (SMD -5.59 [95% CI -9.69, -1.49], p=0.008). Trends to harm were seen using the CB_2 antagonist SR144528 (SMD 0.96, 95% CI -0.32 to 2.24 p=0.14).

There was significant statistical heterogeneity (I^2 56%, p<0.00001, Figure 2) in the all studies analysis.

Drug dose

The effect of drug class dose on infarct volume was analysed to help establish whether there was a dose-response relationship with infarct volume reduction for each class of cannabinoid (Figure 3).

CB_1/CB_2 agonists were significantly effective at numerous doses and showed a bimodal distribution of maximum effect with peaks at 45 mcg/kg (HU-210, SMD -4.16, 95%CI -7.17 to -1.16, p=0.007, n=5 experiments, 50 animals) [10] and 5 mg/kg (WIN 55,212-2, SMD -6.0, 95%CI -9.04 to -2.95, p=0.0001, n=1, 13 animals,[25] Figure 3a). Significant statistical heterogeneity was present (I^2 63%, p<0.00001).

CB_2 ligands were tested between total doses 0.5 mg/kg and 10 mg/kg with peak effect at 5 mg/kg (JWH-133 and O-1966, SMD -2.38, 95%CI -4.06 to -0.71, p=0.005, n=5, 37 animals,[41, 42] Figure 3b). There was no significant statistical heterogeneity.

THC significantly reduced infarct volume at two doses, 10 and 20 mg/kg (SMD -0.95, 95%CI -1.92 to -0.02, p=0.05, n=3, 27 animals; and SMD -2.41, 95%CI -3.29 to -1.53, p<0.00001, n=6, 59 animals, respectively; Figure 3d). A dose-response relationship was observed with CBD, the greatest lesion volume reduction using 6 mg/kg (SMD -1.89, 95%CI -2.7 to -1.07, p<0.00001, n=6, 57 animals [36, 38, 39]). No effect was seen at the greater dose of 10 mg/kg (n=1, 9 animals),[3] Figure 3e.

Peak effect with administration of endocannabinoids was seen at 20 mg/kg (SMD -4.28, 95%CI -6.85 to -1.71, p=0.001, n=2, 16 animals [24, 44]) with significant but less potent effects seen with 30 and 40 mg/kg (Figure 3f). Statistical heterogeneity was evident in the endocannabinoid analysis (I^2 78%, p<0.00001) but not for THC and CBD.

Time of administration

CB_1/CB_2 agonists, assessed up to 8 hours post stroke, revealed a gradual decline in effect size over time, with significant effects seen up to 4-5 hours after insult (Figure 4a). A similar pattern was seen for endocannbinoids but with loss of significant effect as soon as 2-3 hours post stroke (Figure 4f). HU-211 produced significant infarct volume reduction as late as 6 hours post ictus (Figure 4c). Both CBD (up to 6 hours) and THC (up to 4 hours) demonstrated trends to infarct reduction with later administration but there were too few studies to produce significant values at these later time points (Figures 4d and 4e); 17 of 23 experiments using CBD (and 11 of 13 for THC) administered the drug before stroke onset.

Functional outcome and survival

Early neurological outcome improved significantly when evaluated in 55 experiments (590 animals), SMD -1.27 95% CI -1.58 to -0.95, p<0.00001. Late neurological impairment was only assessed in 8 experiments (126 animals) but this still resulted in a significantly improved outcome (p=0.002, Table 2). No effect was seen on survival in 7 experiments (154 animals).

Quality

10 of 34 publications utilised randomisation in their design, 4 reported blinding of outcome assessments, 21 monitored temperature during surgery, 33 measured outcome at 1-3 days and 4 at 7-30 days, 28 measured outcomes other than lesion size, 12 assessed a time window for administration and 16 established dose response effects.

There was no relationship between quality score and lesion volume effect size, Spearman's rho co-efficient -0.113, p=0.18. Likewise, there were no significant differences in effect size when comparing individual components of the scale such as randomisation and blinding of outcome assessment.

Publication bias

Begg's funnel plots were visually analysed to determine the presence or absence of publication bias. For all studies, significant bias was present

(Egger's statistic $p<0.001$, Figure 5H[46]). Significant bias was present in the subgroups $CB_{1/2}$ agonists ($p<0.001$, Figure 5A), CB_2 agonists ($p=0.023$, Figure 5B), HU-211 ($p<0.001$, Figure 5C), and endocannabinoids ($p=0.038$, Figure 5F).

Discussion

This extensive meta-analysis has determined that cannabinoids significantly reduce infarct volume in both transient and permanent models of ischaemia, and improve both early and late functional outcome. Almost twice as many animals were studied in transient (n=945) than permanent (n=519) models and greater infarct volume reductions were seen in permanent models (SMD -1.67 versus -1.41). HU-210, a CB_1/CB_2 agonist, demonstrated the greatest infarct volume reduction and the CB_1/CB_2 agonist group were effective when administered as late as 5 hours post stroke onset. HU-211, a proposed NMDA antagonist and enantiomer of HU-210, was effective up to 6 hours after onset.

The mechanisms of action responsible for the effects of cannabinoids in the pre-clinical setting are multiple but not well understood or always explored within these studies. CB_1 receptors are primarily located in the central nervous system, with activation known to decrease excessive glutamate release, [38] allied excitotoxicity [47] and enhance cerebral blood flow.[48] THC,[36] TAK-937,[33] WIN 55212-2 [19] and HU-210 [10] are protective through CB_1 mediated hypothermia, an effect abolished by warming. CB_2 receptors are expressed predominantly by cells of the immune system but they also display CNS presence, in particular, microglial cells activated during the course of inflammation express CB_2 receptors.[49] Activation of CB_2 receptors results in a decrease in the release of pro-inflammatory cytokines, neutrophil recruitment [9, 41] and leukocyte adhesion to cerebral vessels.[43]

Cannabinoid induced neuroprotection is also likely to be mediated through other receptor targets, though the only proven sites include CB_1,[36, 38] CB_2 [9, 11,

[41] and $5HT_{1A}$.[3, 39] For example, the effects of CBD are not inhibited by CB_1, CB_2 or TRPV1 (transient receptor potential cation channel subfamily V1, or capsaicin receptor) antagonism, but its ability to decrease infarct volume and enhance cerebral blood flow appear to be mediated, at least in part, through $5HT_{1A}$.[3, 39] Other mechanisms, such as anti-inflammatory effects, are yet to be linked to a particular target site, and other known cannabinoid target sites of action such as TRPs and PPARs (peroxisome proliferator-activated receptors) require further exploration. The endocannabinoid PEA is associated with reduced cell death, oedema and inflammation,[18] and OEA is thought to mediate its infarct-reducing effects through PPARα, as the protective effects of OEA were absent in PPAR$\alpha^{-/-}$ mice [40] and inhibited by a PPARα antagonist.[44]

Our systematic review has highlighted many deficiencies in the existing literature that warrant further investigation. It is not apparent that CB_2 antagonists have been tested against mixed CB_1/CB_2 ligands, which is important considering that CB_2 activation is a potential therapeutic target. Furthermore, expression of CB_2 receptors decreases in the first 3 hours after MCAo and then gradually increases by 24 hours.[11] It may, therefore, be of benefit to stimulate CB_2 at later time points; our time-to-treatment analysis only showed a trend to infarct volume reduction at 2-3 hours with CB_2 agonists and there were no experiments extending drug delivery beyond 3 hours. In this review, other cannabinoids show promise with later administration causing significant infarct volume reduction, including CB_1/CB_2 receptor agonists (up to 5 hours) and HU-211 (up to 6 hours). CBD may also be beneficial at later time points with trends to reduce infarct volume as late

as 6 hours but there were too few studies to demonstrate a significant effect; in one study, animal survival was significantly increased even when CBD was administered 3 days following stroke.[35]

The optimal dose of administration for each drug class also remains unclear. It was generally seen that higher doses resulted in a greater degree of infarct volume reduction. Furthermore, questions are raised with regards to the role of CB_1 antagonism; CB_1 agonists mediate their positive effects through the various mechanisms described but CB_1 antagonism with SR141716 used at a high dose (20 mg) also appeared to be beneficial (it was neutral at lower doses). The mechanisms of such an effect are not understood. If CB_1 agonism were detrimental in stroke, the effects of the mixed CB_1/CB_2 agonists should be less than that of the CB_2 specific drugs, although this was not observed. It is more likely that beneficial effects of the CB_1 antagonist at high doses are off-target effects, non-CB_1 mediated responses, as previously suggested for SR141716A.[50]

Further data is also required exploring the effects of cannabinoids in stroke in animals with other co-morbidities, as would occur in humans. For example, only one group have also observed the effects of cannabinoids (TAK-937) in aged and female rats and larger species.[28, 45] Moreover, TAK-937 is the only compound that has examined co-administration with thrombolysis,[28] essential with regards to safety since some neuroprotectants can enhance the risks of rtPA associated haemorrhage.[51] Hypertensive rats have been studied using HU-211 only, [26, 27, 34] but largely data is absent on the effects of cannabinoids in animals with co-morbidities relevant to stroke.[52] It is also clear that neurological assessments of functional outcome at later time points are

lacking with only 8 of 144 experiments (HU-211, CBD, TAK-937) measuring late neuroscores.[26, 35, 45] This is important considering the outcomes in future clinical trials will be related to functional outcome and safety.

There are limited data regarding the safety of cannabinoids in humans and none in the stroke population. Sativex, licenced for use in treating spasticity secondary to Multiple Sclerosis, containing THC and CBD in a 1:1 ratio (2.7mg:2.5mg per 100µl), can commonly cause dizziness, depression, euphoria, gastro-intestinal upset and altered appetite; uncommonly it causes palpitations, tachycardia, hallucinations and suicidal ideation.[53] In a 14-week open label study of 339 patients, 5% discontinued Sativex secondary to treatment related side effects.[54] The psychotropic side effects appear to be mediated through THC CB_1 stimulation and studies using CBD alone, however, indicate that it is very well tolerated; in 3 small studies CBD did not affect heart rate and blood pressure using a single 600mg dose,[55-57] and in regular use for epilepsy (200-300mg), no specific adverse events were reported (4 randomised studies of poor quality, total n=48).[58]

Our paper has a number of limitations affecting interpretation of results, issues that confound many meta-analyses. First, significant heterogeneity is present secondary to the variability in design of individual studies. This is accounted for, in part, by using a random effects model of analysis. Moreover, further heterogeneity is introduced by organising compounds into subgroups; although we have classed the drugs by mechanisms of action, it is likely that many will act on other target sites not identified and therefore statements on efficacy could be an under- or over-estimate. Second, caution must also be taken due to the presence of significant publication bias;[59] our search strategy

may have missed publications in less well known journals; the non-inclusion of some studies means that the estimated treatment effects could be inaccurate. Third, the results also depend on study quality which can also impact report precision; 10 of 34 publications utilised randomisation in their design and only 4 reported blinding of outcome assessments. The impact of various quality items on reported efficacy has been previously assessed;[60] the presence or absence of randomisation to a treatment group, blinding of drug allocation and blinding of outcome assessments were the most powerful determinants of outcome. In contrast, this review did not find any relationship between study quality and efficacy, even when individual components of quality were analysed. However, the absence of some of the parameters in our 'quality' score does not necessarily mean that the experiment was carried out to a poor standard; for example, evaluating timing of outcome assessments is simply expanding the cohort of evidence rather than improving the study quality. Second, it may be that some studies did not report specific components such as randomisation, which may explain why we found no relationship between quality and efficacy. Fourth, many publications would often use an inadequate number of animals in the control arms of the experiments involving multiple comparisons (e.g. comparing several dose-arms to one control group) resulting in smaller control groups in the meta-analysis (it is important not to count the control animals more than once). Moreover, the small group sizes produce imprecise estimates of the variance and, therefore, the SMD. SMD, and not weighted mean difference (WMD), was used in order to merge different scales measuring the same parameter; of 34 publications, 14 measured infarct volume as percentage and 18 used

absolute volume (mm^3). Interpretation of SMD is less intuitive but it has allowed us to include significantly more studies within the analysis.

The failure of multiple neuroprotective agents to be translated into the clinical setting has been extensively highlighted in the literature, [15, 61] hence, evaluating pre-clinical data thoroughly and systematically before progressing to designing human clinical trials is of great importance. Indeed, before moving novel experimental ideas into clinical trials, it is proposed that multi-centre phase III-type preclinical studies are performed (www.dcn.ed.ac.uk/multipart). There are no previous clinical trials using cannabinoids in stroke but positive data from trials using cannabinoids in other neurological diseases already exist.[62] The pleiotropic effects of cannabinoids on the ischaemic penumbra and cerebral vasculature following stroke, combined with their excellent tolerability, make them promising candidates for future treatment.

Conflicts of interest

There were no conflicts of interest during the preparation of the manuscript.

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Titles and Legends to Figures

Figure 1: Record identification process.

Figure 2: Forest plot of the effects of cannabinoids on experimental infarct volume subdivided by drug treatment. Each subgroup is ordered by increasing dose. Time of administration is given where 'pre' represents administration before stroke onset and 'h' the number of hours after.

Figure 3: The effect of cannabinoid drug dose on experimental infarct volume subdivided by drug class. The standardised mean difference (SMD) in infarct volume is plotted against log [dose] for each drug subgroup (a-g). Error bars represent 95% confidence intervals (CI) and values are not significant where they cross zero.

Figure 4: The effect of time of administration on experimental infarct volume subdivided by drug class. The standardised mean difference (SMD) in infarct volume is plotted against time of administration for each drug subgroup (a-f). Error bars represent 95% confidence intervals (CI) and values are not significant where they cross zero.

Figure 5: Funnel plots for all studies (a) and each cannabinoid subgroup (b-h) evaluating publication bias. Standard error of the standardised mean difference (SE (SMD), y-axes) for each study is plotted against its effect size (SMD, horizontal axes)).

Table 1. Description of included studies

Study	Species	Model	Drug	Total Dose	Route	Time of administration	Unit of infarct volume	Time of assessment	STAIR score
Ahmad 2012 [18]	Wistar rats	T 2 h	PEA	10 mg/kg	i.p.	1 and 6 h post	mm^3	24 h	4
Bonfils 2006 [19]	Wistar rats	T 0.5 h	WIN 55,212-2	9 mg/kg/h	i.v.	0.5 h post until 22 h	mm^3	7 d	2
Berger 2004 [20]	Wistar rats	P*	SR141716	1 mg/kg	i.v.	30 min post-onset	mm^3	5 h	3
Belayev 1995a [21]	Wistar rats	T 1.5 h	HU-211	4 mg/kg	i.v.	70 min post-onset	mm^3	72 h	2
Belayev 1995b [22]	Wistar rats	P	HU-211	4 mg/kg	i.v.	30 min post-onset	n/a	n/a	2
Garg 2010 [23]									
Experiments 1-4	Spr-Dawley rats	T 1.5 h	PEA	10 mg/kg	i.p.	Pre, 0, 2 or 3 h post	%	24 h	
Garg 2011 [24]									
Experiment 1	Spr-Dawley rats	T 1.5	Lauroylethanolamide	10 mg/kg	i.p.	Pre onset	%	24 h	4
Experiment 2-3			Linoleoylethanolamide	10 or 20 mg/kg					
Hayakawa 2004 [36]									
Experiment 1	ddY mice	T 4 h	CBD	6 mg/kg	i.p.	Pre-onset	mm^3	24 h	3
Experiment 2			THC	20 mg/kg					
Hayakawa 2007a [12]	ddY mice	T 4 h	THC	20 mg/kg	i.p.	Pre-onset	mm^3	24 h	3
Hayakawa 2007b [39]									
Experiment 1-3	ddY mice	T 4 h	THC	2, 6, 20 mg/kg		Pre-onset	mm^3	24 h	3
Experiment 4-6			CBD	0.2, 2, 6 mg/kg	i.p.				
Experiment 7			SR141716	1 mg/kg					
Hayakawa 2007c [38]									
Experiment 1-3	ddY mice	T 4 h	CBD	0.2, 2, 6 mg/kg	i.p.	Pre-onset	mm^3	24 h	5
Experiment 4-6			THC	2, 6, 20 mg/kg		Pre-onset		24 h	
Experiment 7&9			CBD	6 mg/kg		Pre-onset		24&72 h	
Experiment 8&15			THC	20 mg/kg		Pre-onset		24&72 h	
Experiment 10-14			CBD	3 mg/kg		Pre-onset, 3 h post, at reperfusion, 1 h or 2 h post-reperfusion		24 h	
Experiment 16-18			THC	10 mg/kg		Pre-onset, 3h, reperfusion		24 h	
Experiment 19			SR141716	1 mg/kg		Pre-onset			
Hayakawa 2008 [37]									
Experiment 1-3	ddY mice	T 4 h	CBD	0.1, 1, 3 mg/kg	i.p.	Pre-onset	mm^3	24 h	4
Experiment 4			SR141716	1 mg/kg					
Hayakawa 2009 [35]									
Experiment 1-3	ddY mice	T 4 h	CBD	3 mg/kg	i.p.	Days 1, 3 or 5	n/a	-	4
Hu 2010 [25]									
Experiment 1-3	Spr-Dawley rats	T 2 h	WIN 55,212-2	1, 3 or 5 mg/kg	i.p.	Pre-onset 1, 3 or 5 days	%	72 h	6
Lavie 2001 [26]									
Experiment 1-3	Hypertensive rats	P	HU-211	4.5 mg/kg	i.v.	1, 3 or 6 h post-onset	%	24 h	6
Experiment 4-6						1, 3 or 6 h post onset		30 d	
Leker 1999 [27]	Hypertensive rats	P	HU-211	4 mg/kg	i.v.	1 h post-onset	%	24 h	2
Leker 2003 [10]									
Experiment 1-4	Sp-Dawley rats	P	HU-210	5, 10, 30, 45 μg/kg	i.v.	1 h post-onset	%	72 h	5
Experiment 5-8				45 μg/kg		1, 2, 4, or 6 h post-onset			
Mishima 2005 [3]									
Experiment 1-4	ddY mice	T 4 h	CBD	0.2, 2, 6, or 20 mg/kg	i.p.	At pre-onset	mm^3	24 h	4
Experiment 5-6			Abnormal CBD	6 or 20 mg/kg					
Experiment 7-8			Anandamide	6 or 20 mg/kg					
Experiment 9-10			Methanandamide	6 or 20 mg/kg					

Muthian 2004 [5]									
Experiment 1-3	Wistar rats	T 2.5 h	WIN 55,212-2	0.1, 0.3, or 1 mg/kg	i.v.	5 min pre-onset	mm^3	24 h	5
Experiment 4-6			SR141716	0.1, 0.3, or 1 mg/kg					
Experiment 7			LY320153	6 mg/kg					
Murakami 2013 [28]									
Experiment 1-6	Rats	P	TAK-937	30, 100 mcg/kg/h	i.v.	3, 5 or 8 h post, until 24 h	%	48 h	4
Experiment 7-8		P*				1 h post until 24 h			
Experiment 9	Aged rats	P*		100 mcg/kg/hr		1 h post until 24 h			
Murikinati 2010 [9]	C57BL/6 mice	P	JWH-133	1 mg/kg/day	i.v.	4 hours pre-onset	mm^3	3 d	2
Nagayama 1999 [29]									
Experiment 1-4	Spr-Dawley rats	P	WIN 55,212-2	1 mg/kg	i.p.	30 min pre-, 30, 60, or 120	mm^3	24 h	4
Experiment 5			SR141716	1 mg/kg		min post-			
Schomacher 2008 [30]									
Experiment 1-2	Wistar rats	T 1.5 h	PEA	30 or 10 mg/kg	i.p.	30 min post-onset	mm^3	24 h	5
Experiment 3			Anandamide	10 mg/kg					
Sun 2007 [40]	C57BL/6 Mice	T 2 h	OEA	10mg/kg/day	i.p.	-3,-2 & -1 days pre-onset	mm^3	48 h	1
Sun 2013a [31]									
Experiment 1-2	Spr-Dawley rats	P	WIN 55,212-2	1 or 9 mg/kg	i.v.	2 h post-onset	%	24 h	5
Sun 2013b [32]									
Experiment 1-5	Spr-Dawley rats	P	WIN 55,212-2	1, 3 or 9 mg/kg	i.v.	2 h	%	24 h	4
Experiment 6-7			SR141716	1 or 2 mg					
Suzuki 2012a [45]									
Experiment 1-4	Spr-Dawley rats	T 2 h	TAK-937	3, 10, 30 or 100 μg/kg	i.v.	At reperfusion	%	24 h	4
Experiment 5	Cynomolgus monkeys	T 0.5 h		2 μg/kg		30 min post-reperfusion			
Suzuki 2012b [33]	Spr-Dawley rats	T 2 h	TAK-937	100 mcg/kg/h	i.v.	2 h (on reperfusion) for 24 h	mm^3	24 h	2
Teichner 2003 [34]									
Experiment 1-2	Hypertensive rats	P	HU-211	4.5 mg/kg	i.v.	1 h	%	1 and 30 days	4
Zarruk 2012 [41]									
Experiment 1-3	Mice	P	JWH-133	0.5, 1.5 or 5 mg/kg	i.p.	10 min post-onset	%	48 h	6
Experiment 4-5				1.5 mg/kg		10 min or 3 h post-onset			
Zhang 2007 [43]									
Experiment 1 and 3	Mice	T 1 h	O-3853	1 mg/kg	i.v.	1 h pre- or 10 min post-	mm^3	24 h	3
Experiment 2 and 4			O-1966	1 mg/kg		1 h pre- or 10 min post-			
Zhang 2008 [11]									
Experiment 1-2	Mice	T 1 h	O-1966	1 mg/kg		1 h pre-onset	%	24 h	2
Experiment 3-4			SR141716	5, 20 mg/kg	i.v.				
Experiment 5-6			SR144528	5, 20 mg/kg					
Zhang 2009 [42]									
Experiment 1-3	Mice	T 1 h	O-1966	1, 5, or 10 mg/kg	i.p.	1 h pre-onset	%	24 h	5
Experiment 4-6				5 mg/kg		1 h pre-onset, 1 h or 3 h post-reperfusion			
Zhou 2012 [44]						3 d pre-onset or 30, 60, 90,			
Experiement 1-9	Kunming mice	T 1.5 h	OEA	10, 20, 40 mg/kg	oral	150 min post onset	mm^3	24 h	5

*photothrombotic model; T, transient MCAO; P, permanent MCAO; PEA, palmitoylethanolamide; THC, Δ^9-tetrahydrocannabinol; CBD, cannabidiol; OEA, oleoylethanolamide; i.p., intra-peritoneal; i.v., intravenous

Table 2. Change in infarct volume (according to stroke model, species and drug class), motor impairment and survival following administration of any cannabinoid in experimental stroke.

	N° of experiments	N° of animals	SMD [95% CI]	P-value
		Lesion volume		
Stroke model				
Transient	90	945	-1.41 [-1.71, -1.11]	< 0.00001
Permanent	54	519	-1.67 [-2.08, -1.27]	< 0.00001
Species				
Rats	69	786	-1.75 [-2.15, -1.35]	< 0.00001
Mice	74	673	-1.34 [-1.61, -1.06]	< 0.00001
Monkeys	1	14	-0.55 [-1.63, 0.53]	0.32
Drug Class				
Endocannabinoids	25	268	-1.72 [-2.62, -0.82]	0.0002
Synthetic cannabinoids				
Mixed CB_1/CB_2 ligands	41	494	-1.75 [-2.19, -1.31]	< 0.00001
CB_2 ligands	18	162	-1.65 [-2.09, -1.22]	< 0.00001
Abnormal CBD	2	16	-0.56 [-2.08, 0.95]	0.47
HU-211	10	113	-2.90 [-4.24, -1.56]	<0.0001
CB_1 antagonists	12	103	-0.70 [-1.22, -0.18]	0.009
CB_2 antagonists	2	14	0.96 [-0.32, 2.24]	0.14
Phytocannbinoids				
THC	13	115	-1.43 [-2.01, -0.86]	<0.00001
CBD	21	188	-1.20 [-1.63, -0.77]	<0.00001
		Motor Impairment		
Early (24-72hrs) neuro-score	55	590	-1.27 [-1.58, -0.95]	<0.00001
Late (2-4 weeks) neuro-score	8	126	-1.63 [-2.64, -0.62]	0.002
Rotarod (24 hours post IS)	10	86	6.09 [0.7, 11.48]*	0.03
		Survival		
Transient ischaemia	7	154	2.09 (0.39, 11.3)†	0.39

SMD, standardised mean difference; CI, confidence interval; * weighted mean difference (seconds); † odds ratio; IS, ischaemic stroke

Figure 1

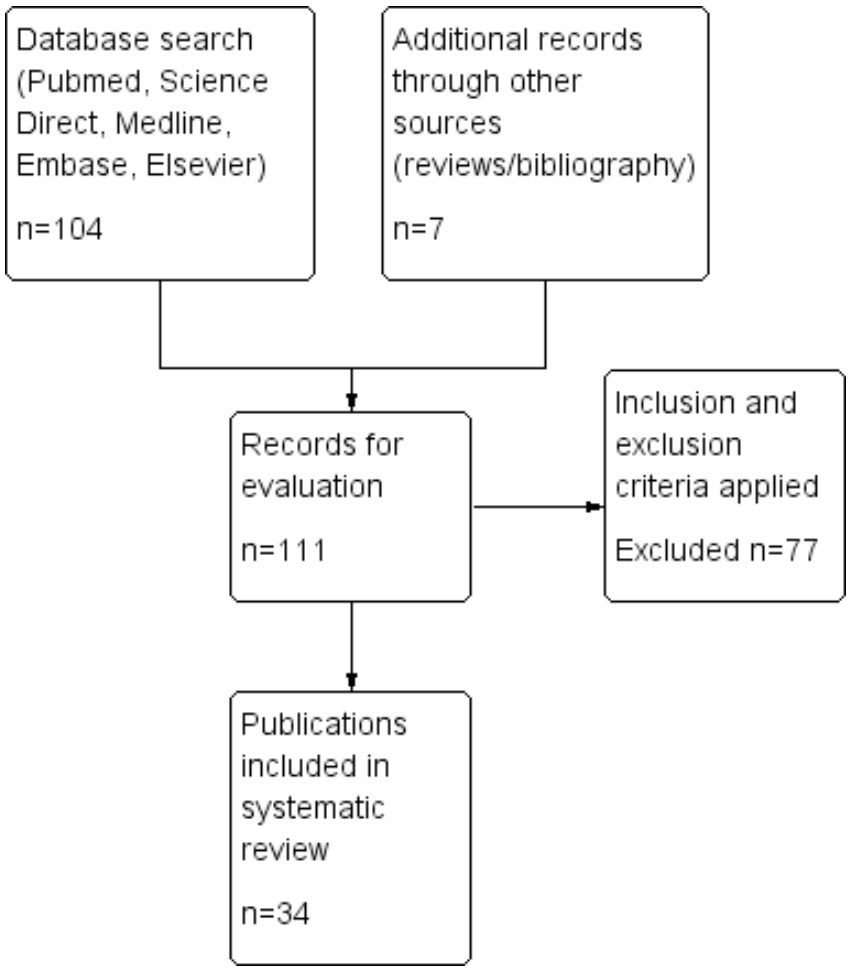
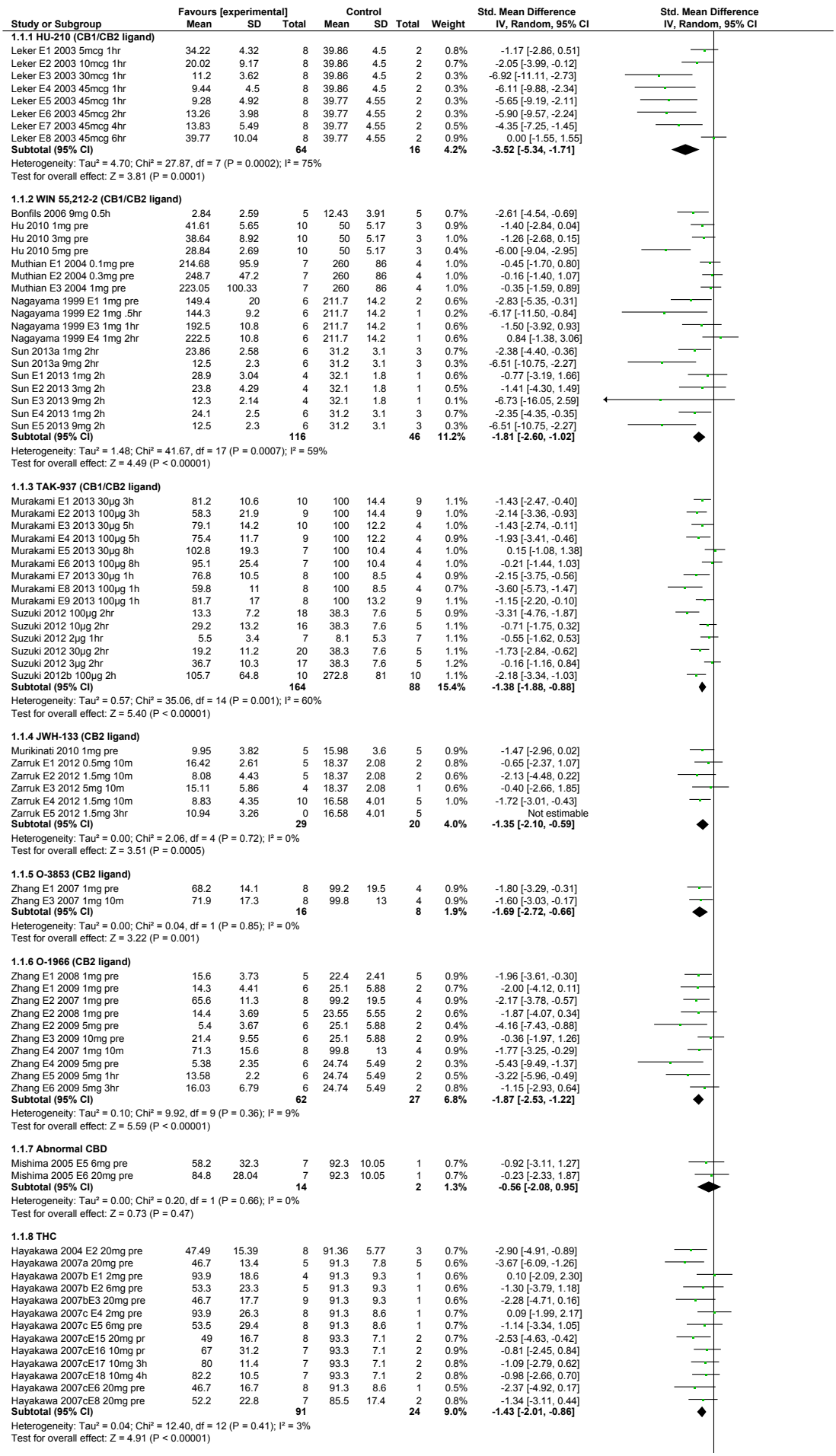

Database search (Pubmed, Science Direct, Medline, Embase, Elsevier)
n=104
Additional records through other sources (reviews/bibliography)
n=7
Records for evaluation
n=111
Inclusion and exclusion criteria applied
Excluded n=77
Publications included in systematic review
n=34

Figure 2


Favours [experimental]
Control
Std. Mean Difference
Std. Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
1.1.1 HU-210 (CB1/CB2 ligand)
Leker E1 2003 5mcg 1hr 34.22 4.32 8 39.86 4.5 2 0.8% -1.17 [-2.86, 0.51]
Leker E2 2003 10mcg 1hr 20.02 9.17 8 39.86 4.5 2 0.7% -2.05 [-3.99, -0.12]
Leker E3 2003 30mcg 1hr 11.2 3.62 8 39.86 4.5 2 0.3% -6.92 [-11.11, -2.73]
Leker E4 2003 45mcg 1hr 9.44 4.5 8 39.86 4.5 2 0.3% -6.11 [-9.88, -2.34]
Leker E5 2003 45mcg 1hr 9.28 4.92 8 39.77 4.55 2 0.3% -5.65 [-9.19, -2.11]
Leker E6 2003 45mcg 2hr 13.26 3.98 8 39.77 4.55 2 0.3% -5.90 [-9.57, -2.24]
Leker E7 2003 45mcg 4hr 13.83 5.49 8 39.77 4.55 2 0.5% -4.35 [-7.25, -1.45]
Leker E8 2003 45mcg 6hr 39.77 10.04 8 39.77 4.55 2 0.9% 0.00 [-1.55, 1.55]
Subtotal (95% CI) 64 16 4.2% -3.52 [-5.34, -1.71]
Heterogeneity: Tau² = 4.70; Chi² = 27.87, df = 7 (P = 0.0002); I² = 75%
Test for overall effect: Z = 3.81 (P = 0.0001)
1.1.2 WIN 55,212-2 (CB1/CB2 ligand)
Bonfils 2006 9mg 0.5h 2.84 2.59 5 12.43 3.91 5 0.7% -2.61 [-4.54, -0.69]
Hu 2010 1mg pre 41.61 5.65 10 50 5.17 3 0.9% -1.40 [-2.84, 0.04]
Hu 2010 3mg pre 38.64 8.92 10 50 5.17 3 1.0% -1.26 [-2.68, 0.15]
Hu 2010 5mg pre 28.84 2.69 10 50 5.17 3 0.4% -6.00 [-9.04, -2.95]
Muthian E1 2004 0.1mg pre 214.68 95.9 7 260 86 4 1.0% -0.45 [-1.70, 0.80]
Muthian E2 2004 0.3mg pre 248.7 47.2 7 260 86 4 1.0% -0.16 [-1.40, 1.07]
Muthian E3 2004 1mg pre 223.05 100.33 7 260 86 4 1.0% -0.35 [-1.59, 0.89]
Nagayama 1999 E1 1mg pre 149.4 20 6 211.7 14.2 2 0.6% -2.83 [-5.35, -0.31]
Nagayama 1999 E2 1mg .5hr 144.3 9.2 6 211.7 14.2 1 0.2% -6.17 [-11.50, -0.84]
Nagayama 1999 E3 1mg 1hr 192.5 10.8 6 211.7 14.2 1 0.6% -1.50 [-3.92, 0.93]
Nagayama 1999 E4 1mg 2hr 222.5 10.8 6 211.7 14.2 1 0.6% 0.84 [-1.38, 3.06]
Sun 2013a 1mg 2hr 23.86 2.58 6 31.2 3.1 3 0.7% -2.38 [-4.40, -0.36]
Sun 2013a 9mg 2hr 12.5 2.3 6 31.2 3.1 3 0.3% -6.51 [-10.75, -2.27]
Sun E1 2013 1mg 2h 28.9 3.04 4 32.1 1.8 1 0.6% -0.77 [-3.19, 1.66]
Sun E2 2013 3mg 2h 23.8 4.29 4 32.1 1.8 1 0.5% -1.41 [-4.30, 1.49]
Sun E3 2013 9mg 2h 12.3 2.14 4 32.1 1.8 1 0.1% -6.73 [-16.05, 2.59]
Sun E4 2013 1mg 2h 24.1 2.5 6 31.2 3.1 3 0.7% -2.35 [-4.35, -0.35]
Sun E5 2013 9mg 2h 12.5 2.3 6 31.2 3.1 3 0.3% -6.51 [-10.75, -2.27]
Subtotal (95% CI) 116 46 11.2% -1.81 [-2.60, -1.02]
Heterogeneity: Tau² = 1.48; Chi² = 41.67, df = 17 (P = 0.0007); I² = 59%
Test for overall effect: Z = 4.49 (P < 0.00001)
1.1.3 TAK-937 (CB1/CB2 ligand)
Murakami E1 2013 30µg 3h 81.2 10.6 10 100 14.4 9 1.1% -1.43 [-2.47, -0.40]
Murakami E2 2013 100µg 3h 58.3 21.9 9 100 14.4 9 1.0% -2.14 [-3.36, -0.93]
Murakami E3 2013 30µg 5h 79.1 14.2 10 100 12.2 4 1.0% -1.43 [-2.74, -0.11]
Murakami E4 2013 100µg 5h 75.4 11.7 9 100 12.2 4 0.9% -1.93 [-3.41, -0.46]
Murakami E5 2013 30µg 8h 102.8 19.3 7 100 10.4 4 1.0% 0.15 [-1.08, 1.38]
Murakami E6 2013 100µg 8h 95.1 25.4 7 100 10.4 4 1.0% -0.21 [-1.44, 1.03]
Murakami E7 2013 30µg 1h 76.8 10.5 8 100 8.5 4 0.9% -2.15 [-3.75, -0.56]
Murakami E8 2013 100µg 1h 59.8 11 8 100 8.5 4 0.7% -3.60 [-5.73, -1.47]
Murakami E9 2013 100µg 1h 81.7 17 8 100 13.2 9 1.1% -1.15 [-2.20, -0.10]
Suzuki 2012 100µg 2hr 13.3 7.2 18 38.3 7.6 5 0.9% -3.31 [-4.76, -1.87]
Suzuki 2012 10µg 2hr 29.2 13.2 16 38.3 7.6 5 1.1% -0.71 [-1.75, 0.32]
Suzuki 2012 2µg 1hr 5.5 3.4 7 8.1 5.3 7 1.1% -0.55 [-1.62, 0.53]
Suzuki 2012 30µg 2hr 19.2 11.2 20 38.3 7.6 5 1.1% -1.73 [-2.84, -0.62]
Suzuki 2012 3µg 2hr 36.7 10.3 17 38.3 7.6 5 1.2% -0.16 [-1.16, 0.84]
Suzuki 2012b 100µg 2h 105.7 64.8 10 272.8 81 10 1.1% -2.18 [-3.34, -1.03]
Subtotal (95% CI) 164 88 15.4% -1.38 [-1.88, -0.88]
Heterogeneity: Tau² = 0.57; Chi² = 35.06, df = 14 (P = 0.001); I² = 60%
Test for overall effect: Z = 5.40 (P < 0.00001)
1.1.4 JWH-133 (CB2 ligand)
Murikinati 2010 1mg pre 9.95 3.82 5 15.98 3.6 5 0.9% -1.47 [-2.96, 0.02]
Zarruk E1 2012 0.5mg 10m 16.42 2.61 5 18.37 2.08 2 0.8% -0.65 [-2.37, 1.07]
Zarruk E2 2012 1.5mg 10m 8.08 4.43 5 18.37 2.08 2 0.6% -2.13 [-4.48, 0.22]
Zarruk E3 2012 5mg 10m 15.11 5.86 4 18.37 2.08 1 0.6% -0.40 [-2.66, 1.85]
Zarruk E4 2012 1.5mg 10m 8.83 4.35 10 16.58 4.01 5 1.0% -1.72 [-3.01, -0.43]
Zarruk E5 2012 1.5mg 3hr 10.94 3.26 0 16.58 4.01 5 Not estimable
Subtotal (95% CI) 29 20 4.0% -1.35 [-2.10, -0.59]
Heterogeneity: Tau² = 0.00; Chi² = 2.06, df = 4 (P = 0.72); I² = 0%
Test for overall effect: Z = 3.51 (P = 0.0005)
1.1.5 O-3853 (CB2 ligand)
Zhang E1 2007 1mg pre 68.2 14.1 8 99.2 19.5 4 0.9% -1.80 [-3.29, -0.31]
Zhang E3 2007 1mg 10m 71.9 17.3 8 99.8 13 4 0.9% -1.60 [-3.03, -0.17]
Subtotal (95% CI) 16 8 1.9% -1.69 [-2.72, -0.66]
Heterogeneity: Tau² = 0.00; Chi² = 0.04, df = 1 (P = 0.85); I² = 0%
Test for overall effect: Z = 3.22 (P = 0.001)
1.1.6 O-1966 (CB2 ligand)
Zhang E1 2008 1mg pre 15.6 3.73 5 22.4 2.41 5 0.9% -1.96 [-3.61, -0.30]
Zhang E1 2009 1mg pre 14.3 4.41 6 25.1 5.88 2 0.7% -2.00 [-4.12, 0.11]
Zhang E2 2007 1mg pre 65.6 11.3 8 99.2 19.5 4 0.9% -2.17 [-3.78, -0.57]
Zhang E2 2008 1mg pre 14.4 3.69 5 23.55 5.55 2 0.6% -1.87 [-4.07, 0.34]
Zhang E2 2009 5mg pre 5.4 3.67 6 25.1 5.88 2 0.4% -4.16 [-7.43, -0.88]
Zhang E3 2009 10mg pre 21.4 9.55 6 25.1 5.88 2 0.9% -0.36 [-1.97, 1.26]
Zhang E4 2007 1mg 10m 71.3 15.6 8 99.8 13 4 0.9% -1.77 [-3.25, -0.29]
Zhang E4 2009 5mg pre 5.38 2.35 6 24.74 5.49 2 0.3% -5.43 [-9.49, -1.37]
Zhang E5 2009 5mg 1hr 13.58 2.2 6 24.74 5.49 2 0.5% -3.22 [-5.96, -0.49]
Zhang E6 2009 5mg 3hr 16.03 6.79 6 24.74 5.49 2 0.8% -1.15 [-2.93, 0.64]
Subtotal (95% CI) 62 27 6.8% -1.87 [-2.53, -1.22]
Heterogeneity: Tau² = 0.10; Chi² = 9.92, df = 9 (P = 0.36); I² = 9%
Test for overall effect: Z = 5.59 (P < 0.00001)
1.1.7 Abnormal CBD
Mishima 2005 E5 6mg pre 58.2 32.3 7 92.3 10.05 1 0.7% -0.92 [-3.11, 1.27]
Mishima 2005 E6 20mg pre 84.8 28.04 7 92.3 10.05 1 0.7% -0.23 [-2.33, 1.87]
Subtotal (95% CI) 14 2 1.3% -0.56 [-2.08, 0.95]
Heterogeneity: Tau² = 0.00; Chi² = 0.20, df = 1 (P = 0.66); I² = 0%
Test for overall effect: Z = 0.73 (P = 0.47)
1.1.8 THC
Hayakawa 2004 E2 20mg pre 47.49 15.39 8 91.36 5.77 3 0.7% -2.90 [-4.91, -0.89]
Hayakawa 2007a 20mg pre 46.7 13.4 5 91.3 7.8 5 0.6% -3.67 [-6.09, -1.26]
Hayakawa 2007b E1 2mg pre 93.9 18.6 4 91.3 9.3 1 0.6% 0.10 [-2.09, 2.30]
Hayakawa 2007b E2 6mg pre 53.3 23.3 5 91.3 9.3 1 0.6% -1.30 [-3.79, 1.18]
Hayakawa 2007bE3 20mg pre 46.7 17.7 9 91.3 9.3 1 0.6% -2.28 [-4.71, 0.16]
Hayakawa 2007c E4 2mg pre 93.9 26.3 8 91.3 8.6 1 0.7% 0.09 [-1.99, 2.17]
Hayakawa 2007c E5 6mg pre 53.5 29.4 8 91.3 8.6 1 0.7% -1.14 [-3.34, 1.05]
Hayakawa 2007cE15 20mg pr 49 16.7 8 93.3 7.1 2 0.7% -2.53 [-4.63, -0.42]
Hayakawa 2007cE16 10mg pr 67 31.2 7 93.3 7.1 2 0.9% -0.81 [-2.45, 0.84]
Hayakawa 2007cE17 10mg 3h 80 11.4 7 93.3 7.1 2 0.8% -1.09 [-2.79, 0.62]
Hayakawa 2007cE18 10mg 4h 82.2 10.5 7 93.3 7.1 2 0.8% -0.98 [-2.66, 0.70]
Hayakawa 2007cE6 20mg pre 46.7 16.7 8 91.3 8.6 1 0.5% -2.37 [-4.92, 0.17]
Hayakawa 2007cE8 20mg pre 52.2 22.8 7 85.5 17.4 2 0.8% -1.34 [-3.11, 0.44]
Subtotal (95% CI) 91 24 9.0% -1.43 [-2.01, -0.86]
Heterogeneity: Tau² = 0.04; Chi² = 12.40, df = 12 (P = 0.41); I² = 3%
Test for overall effect: Z = 4.91 (P < 0.00001)

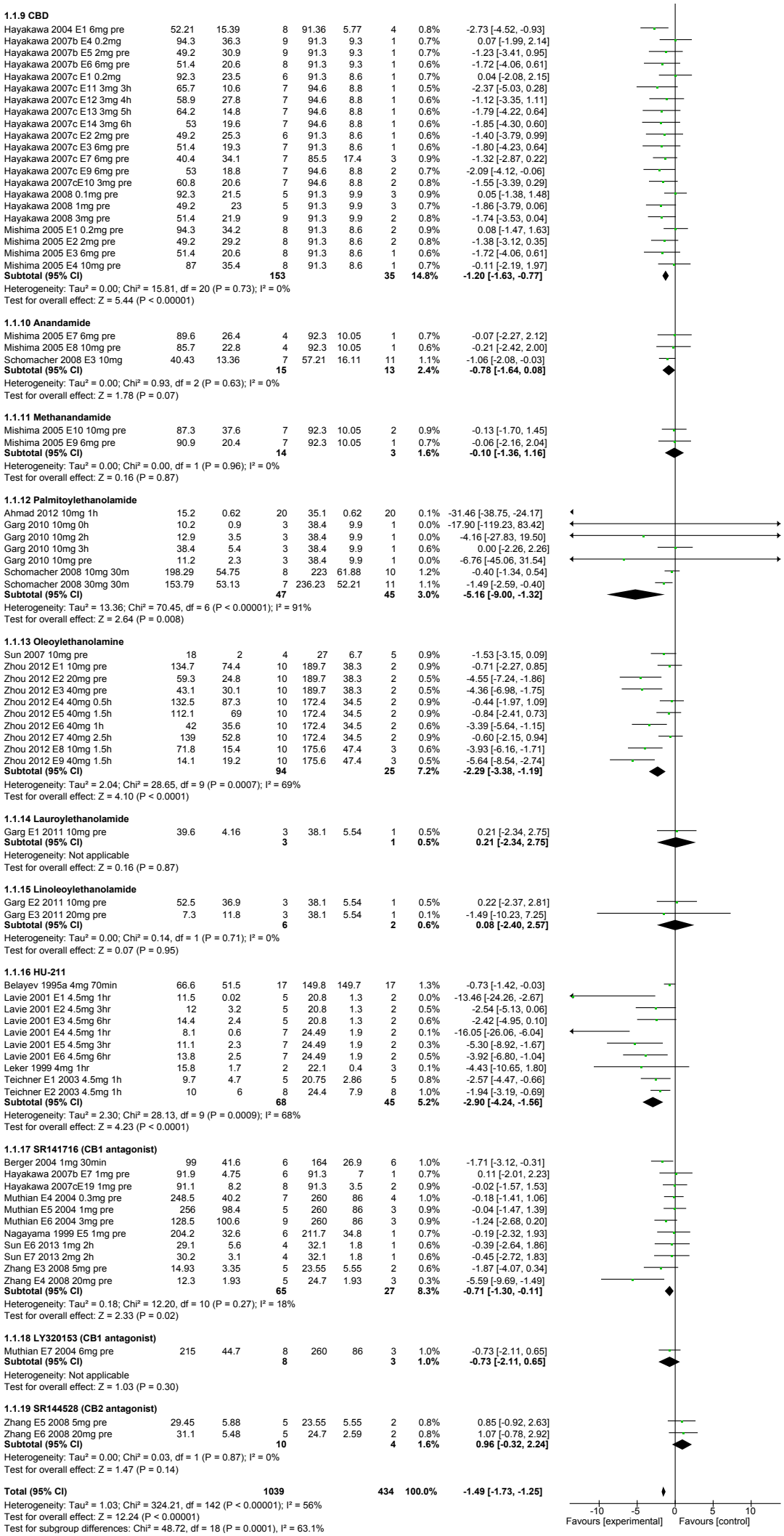

1.1.9 CBD
Hayakawa 2004 E1 6mg pre 52.21 15.39 8 91.36 5.77 4 0.8% -2.73 [-4.52, -0.93]
Hayakawa 2007b E4 0.2mg 94.3 36.3 9 91.3 9.3 1 0.7% 0.07 [-1.99, 2.14]
Hayakawa 2007b E5 2mg pre 49.2 30.9 9 91.3 9.3 1 0.7% -1.23 [-3.41, 0.95]
Hayakawa 2007b E6 6mg pre 51.4 20.6 8 91.3 9.3 1 0.6% -1.72 [-4.06, 0.61]
Hayakawa 2007c E1 0.2mg 92.3 23.5 6 91.3 8.6 1 0.7% 0.04 [-2.08, 2.15]
Hayakawa 2007c E11 3mg 3h 65.7 10.6 7 94.6 8.8 1 0.5% -2.37 [-5.03, 0.28]
Hayakawa 2007c E12 3mg 4h 58.9 27.8 7 94.6 8.8 1 0.6% -1.12 [-3.35, 1.11]
Hayakawa 2007c E13 3mg 5h 64.2 14.8 7 94.6 8.8 1 0.6% -1.79 [-4.22, 0.64]
Hayakawa 2007c E14 3mg 6h 53 19.6 7 94.6 8.8 1 0.6% -1.85 [-4.30, 0.60]
Hayakawa 2007c E2 2mg pre 49.2 25.3 6 91.3 8.6 1 0.6% -1.40 [-3.79, 0.99]
Hayakawa 2007c E3 6mg pre 51.4 19.3 7 91.3 8.6 1 0.6% -1.80 [-4.23, 0.64]
Hayakawa 2007c E7 6mg pre 40.4 34.1 7 85.5 17.4 3 0.9% -1.32 [-2.87, 0.22]
Hayakawa 2007c E9 6mg pre 53 18.8 7 94.6 8.8 2 0.7% -2.09 [-4.12, -0.06]
Hayakawa 2007cE10 3mg pre 60.8 20.6 7 94.6 8.8 2 0.8% -1.55 [-3.39, 0.29]
Hayakawa 2008 0.1mg pre 92.3 21.5 5 91.3 9.9 3 0.9% 0.05 [-1.38, 1.48]
Hayakawa 2008 1mg pre 49.2 23 5 91.3 9.9 3 0.7% -1.86 [-3.79, 0.06]
Hayakawa 2008 3mg pre 51.4 21.9 9 91.3 9.9 2 0.8% -1.74 [-3.53, 0.04]
Mishima 2005 E1 0.2mg pre 94.3 34.2 8 91.3 8.6 2 0.9% 0.08 [-1.47, 1.63]
Mishima 2005 E2 2mg pre 49.2 29.2 8 91.3 8.6 2 0.8% -1.38 [-3.12, 0.35]
Mishima 2005 E3 6mg pre 51.4 20.6 8 91.3 8.6 1 0.6% -1.72 [-4.06, 0.61]
Mishima 2005 E4 10mg pre 87 35.4 8 91.3 8.6 1 0.7% -0.11 [-2.19, 1.97]
Subtotal (95% CI) 153 35 14.8% -1.20 [-1.63, -0.77]
Heterogeneity: Tau² = 0.00; Chi² = 15.81, df = 20 (P = 0.73); I² = 0%
Test for overall effect: Z = 5.44 (P < 0.00001)
1.1.10 Anandamide
Mishima 2005 E7 6mg pre 89.6 26.4 4 92.3 10.05 1 0.7% -0.07 [-2.27, 2.12]
Mishima 2005 E8 10mg pre 85.7 22.8 4 92.3 10.05 1 0.6% -0.21 [-2.42, 2.00]
Schomacher 2008 E3 10mg 40.43 13.36 7 57.21 16.11 11 1.1% -1.06 [-2.08, -0.03]
Subtotal (95% CI) 15 13 2.4% -0.78 [-1.64, 0.08]
Heterogeneity: Tau² = 0.00; Chi² = 0.93, df = 2 (P = 0.63); I² = 0%
Test for overall effect: Z = 1.78 (P = 0.07)
1.1.11 Methanandamide
Mishima 2005 E10 10mg pre 87.3 37.6 7 92.3 10.05 2 0.9% -0.13 [-1.70, 1.45]
Mishima 2005 E9 6mg pre 90.9 20.4 7 92.3 10.05 1 0.7% -0.06 [-2.16, 2.04]
Subtotal (95% CI) 14 3 1.6% -0.10 [-1.36, 1.16]
Heterogeneity: Tau² = 0.00; Chi² = 0.00, df = 1 (P = 0.96); I² = 0%
Test for overall effect: Z = 0.16 (P = 0.87)
1.1.12 Palmitoylethanolamide
Ahmad 2012 10mg 1h 15.2 0.62 20 35.1 0.62 20 0.1% -31.46 [-38.75, -24.17]
Garg 2010 10mg 0h 10.2 0.9 3 38.4 9.9 1 0.0% -17.90 [-119.23, 83.42]
Garg 2010 10mg 2h 12.9 3.5 3 38.4 9.9 1 0.0% -4.16 [-27.83, 19.50]
Garg 2010 10mg 3h 38.4 5.4 3 38.4 9.9 1 0.6% 0.00 [-2.26, 2.26]
Garg 2010 10mg pre 11.2 2.3 3 38.4 9.9 1 0.0% -6.76 [-45.06, 31.54]
Schomacher 2008 10mg 30m 198.29 54.75 8 223 61.88 10 1.2% -0.40 [-1.34, 0.54]
Schomacher 2008 30mg 30m 153.79 53.13 7 236.23 52.21 11 1.1% -1.49 [-2.59, -0.40]
Subtotal (95% CI) 47 45 3.0% -5.16 [-9.00, -1.32]
Heterogeneity: Tau² = 13.36; Chi² = 70.45, df = 6 (P < 0.00001); I² = 91%
Test for overall effect: Z = 2.64 (P = 0.008)
1.1.13 Oleoylethanolamine
Sun 2007 10mg pre 18 2 4 27 6.7 5 0.9% -1.53 [-3.15, 0.09]
Zhou 2012 E1 10mg pre 134.7 74.4 10 189.7 38.3 2 0.9% -0.71 [-2.27, 0.85]
Zhou 2012 E2 20mg pre 59.3 24.8 10 189.7 38.3 2 0.5% -4.55 [-7.24, -1.86]
Zhou 2012 E3 40mg pre 43.1 30.1 10 189.7 38.3 2 0.5% -4.36 [-6.98, -1.75]
Zhou 2012 E4 40mg 0.5h 132.5 87.3 10 172.4 34.5 2 0.9% -0.44 [-1.97, 1.09]
Zhou 2012 E5 40mg 1.5h 112.1 69 10 172.4 34.5 2 0.9% -0.84 [-2.41, 0.73]
Zhou 2012 E6 40mg 1h 42 35.6 10 172.4 34.5 2 0.6% -3.39 [-5.64, -1.15]
Zhou 2012 E7 40mg 2.5h 139 52.8 10 172.4 34.5 2 0.9% -0.60 [-2.15, 0.94]
Zhou 2012 E8 10mg 1.5h 71.8 15.4 10 175.6 47.4 3 0.6% -3.93 [-6.16, -1.71]
Zhou 2012 E9 40mg 1.5h 14.1 19.2 10 175.6 47.4 3 0.5% -5.64 [-8.54, -2.74]
Subtotal (95% CI) 94 25 7.2% -2.29 [-3.38, -1.19]
Heterogeneity: Tau² = 2.04; Chi² = 28.65, df = 9 (P = 0.0007); I² = 69%
Test for overall effect: Z = 4.10 (P < 0.0001)
1.1.14 Lauroylethanolamide
Garg E1 2011 10mg pre 39.6 4.16 3 38.1 5.54 1 0.5% 0.21 [-2.34, 2.75]
Subtotal (95% CI) 3 1 0.5% 0.21 [-2.34, 2.75]
Heterogeneity: Not applicable
Test for overall effect: Z = 0.16 (P = 0.87)
1.1.15 Linoleoylethanolamide
Garg E2 2011 10mg pre 52.5 36.9 3 38.1 5.54 1 0.5% 0.22 [-2.37, 2.81]
Garg E3 2011 20mg pre 7.3 11.8 3 38.1 5.54 1 0.1% -1.49 [-10.23, 7.25]
Subtotal (95% CI) 6 2 0.6% 0.08 [-2.40, 2.57]
Heterogeneity: Tau² = 0.00; Chi² = 0.14, df = 1 (P = 0.71); I² = 0%
Test for overall effect: Z = 0.07 (P = 0.95)
1.1.16 HU-211
Belayev 1995a 4mg 70min 66.6 51.5 17 149.8 149.7 17 1.3% -0.73 [-1.42, -0.03]
Lavie 2001 E1 4.5mg 1hr 11.5 0.02 5 20.8 1.3 2 0.0% -13.46 [-24.26, -2.67]
Lavie 2001 E2 4.5mg 3hr 12 3.2 5 20.8 1.3 2 0.5% -2.54 [-5.13, 0.06]
Lavie 2001 E3 4.5mg 6hr 14.4 2.4 5 20.8 1.3 2 0.6% -2.42 [-4.95, 0.10]
Lavie 2001 E4 4.5mg 1hr 8.1 0.6 7 24.49 1.9 2 0.1% -16.05 [-26.06, -6.04]
Lavie 2001 E5 4.5mg 3hr 11.1 2.3 7 24.49 1.9 2 0.3% -5.30 [-8.92, -1.67]
Lavie 2001 E6 4.5mg 6hr 13.8 2.5 7 24.49 1.9 2 0.5% -3.92 [-6.80, -1.04]
Leker 1999 4mg 1hr 15.8 1.7 2 22.1 0.4 3 0.1% -4.43 [-10.65, 1.80]
Teichner E1 2003 4.5mg 1h 9.7 4.7 5 20.75 2.86 5 0.8% -2.57 [-4.47, -0.66]
Teichner E2 2003 4.5mg 1h 10 6 8 24.4 7.9 8 1.0% -1.94 [-3.19, -0.69]
Subtotal (95% CI) 68 45 5.2% -2.90 [-4.24, -1.56]
Heterogeneity: Tau² = 2.30; Chi² = 28.13, df = 9 (P = 0.0009); I² = 68%
Test for overall effect: Z = 4.23 (P < 0.0001)
1.1.17 SR141716 (CB1 antagonist)
Berger 2004 1mg 30min 99 41.6 6 164 26.9 6 1.0% -1.71 [-3.12, -0.31]
Hayakawa 2007b E7 1mg pre 91.9 4.75 6 91.3 7 1 0.7% 0.11 [-2.01, 2.23]
Hayakawa 2007cE19 1mg pre 91.1 8.2 8 91.3 3.5 2 0.9% -0.02 [-1.57, 1.53]
Muthian E4 2004 0.3mg pre 248.5 40.2 7 260 86 4 1.0% -0.18 [-1.41, 1.06]
Muthian E5 2004 1mg pre 256 98.4 5 260 86 3 0.9% -0.04 [-1.47, 1.39]
Muthian E6 2004 3mg pre 128.5 100.6 9 260 86 3 0.9% -1.24 [-2.68, 0.20]
Nagayama 1999 E5 1mg pre 204.2 32.6 6 211.7 34.8 1 0.7% -0.19 [-2.32, 1.93]
Sun E6 2013 1mg 2h 29.1 5.6 4 32.1 1.8 1 0.6% -0.39 [-2.64, 1.86]
Sun E7 2013 2mg 2h 30.2 3.1 4 32.1 1.8 1 0.6% -0.45 [-2.72, 1.83]
Zhang E3 2008 5mg pre 14.93 3.35 5 23.55 5.55 2 0.6% -1.87 [-4.07, 0.34]
Zhang E4 2008 20mg pre 12.3 1.93 5 24.7 1.93 3 0.3% -5.59 [-9.69, -1.49]
Subtotal (95% CI) 65 27 8.3% -0.71 [-1.30, -0.11]
Heterogeneity: Tau² = 0.18; Chi² = 12.20, df = 10 (P = 0.27); I² = 18%
Test for overall effect: Z = 2.33 (P = 0.02)
1.1.18 LY320153 (CB1 antagonist)
Muthian E7 2004 6mg pre 215 44.7 8 260 86 3 1.0% -0.73 [-2.11, 0.65]
Subtotal (95% CI) 8 3 1.0% -0.73 [-2.11, 0.65]
Heterogeneity: Not applicable
Test for overall effect: Z = 1.03 (P = 0.30)
1.1.19 SR144528 (CB2 antagonist)
Zhang E5 2008 5mg pre 29.45 5.88 5 23.55 5.55 2 0.8% 0.85 [-0.92, 2.63]
Zhang E6 2008 20mg pre 31.1 5.48 5 24.7 2.59 2 0.8% 1.07 [-0.78, 2.92]
Subtotal (95% CI) 10 4 1.6% 0.96 [-0.32, 2.24]
Heterogeneity: Tau² = 0.00; Chi² = 0.03, df = 1 (P = 0.87); I² = 0%
Test for overall effect: Z = 1.47 (P = 0.14)
Total (95% CI) 1039 434 100.0% -1.49 [-1.73, -1.25]
Heterogeneity: Tau² = 1.03; Chi² = 324.21, df = 142 (P < 0.00001); I² = 56%
Test for overall effect: Z = 12.24 (P < 0.00001)
Test for subgroup differences: Chi² = 48.72, df = 18 (P = 0.0001), I² = 63.1%
-10 -5 0 5 10
Favours [experimental] Favours [control]

Figure 3

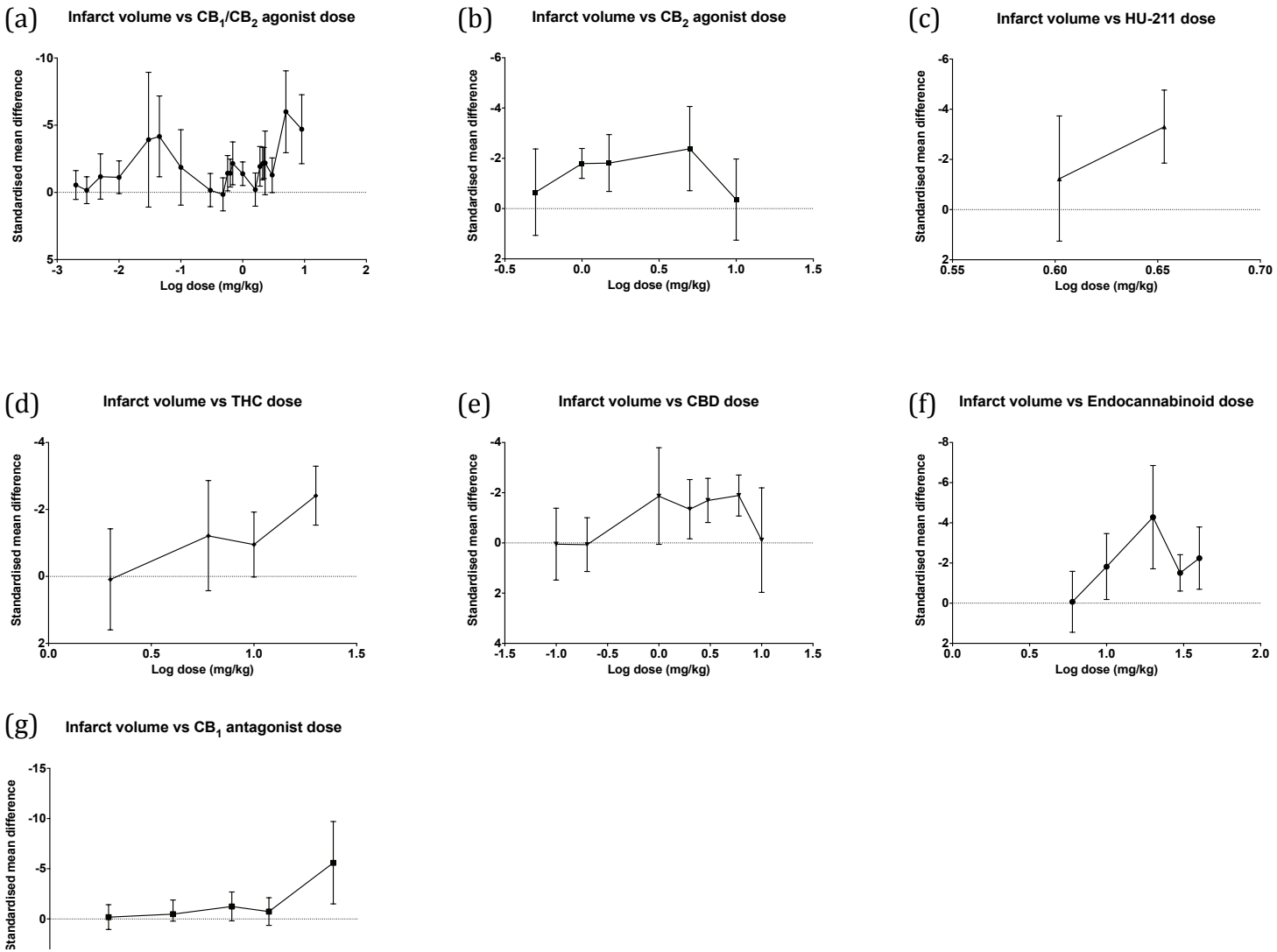

(a)
Infarct volume vs CB1/CB2 agonist dose
Standardised mean difference
Log dose (mg/kg)
(b)
Infarct volume vs CB2 agonist dose
Standardised mean difference
Log dose (mg/kg)
(c)
Infarct volume vs HU-211 dose
Standardised mean difference
Log dose (mg/kg)
(d)
Infarct volume vs THC dose
Standardised mean difference
Log dose (mg/kg)
(e)
Infarct volume vs CBD dose
Standardised mean difference
Log dose (mg/kg)
(f)
Infarct volume vs Endocannabinoid dose
Standardised mean difference
Log dose (mg/kg)
(g)
Infarct volume vs CB1 antagonist dose
Standardised mean difference

Figure 4

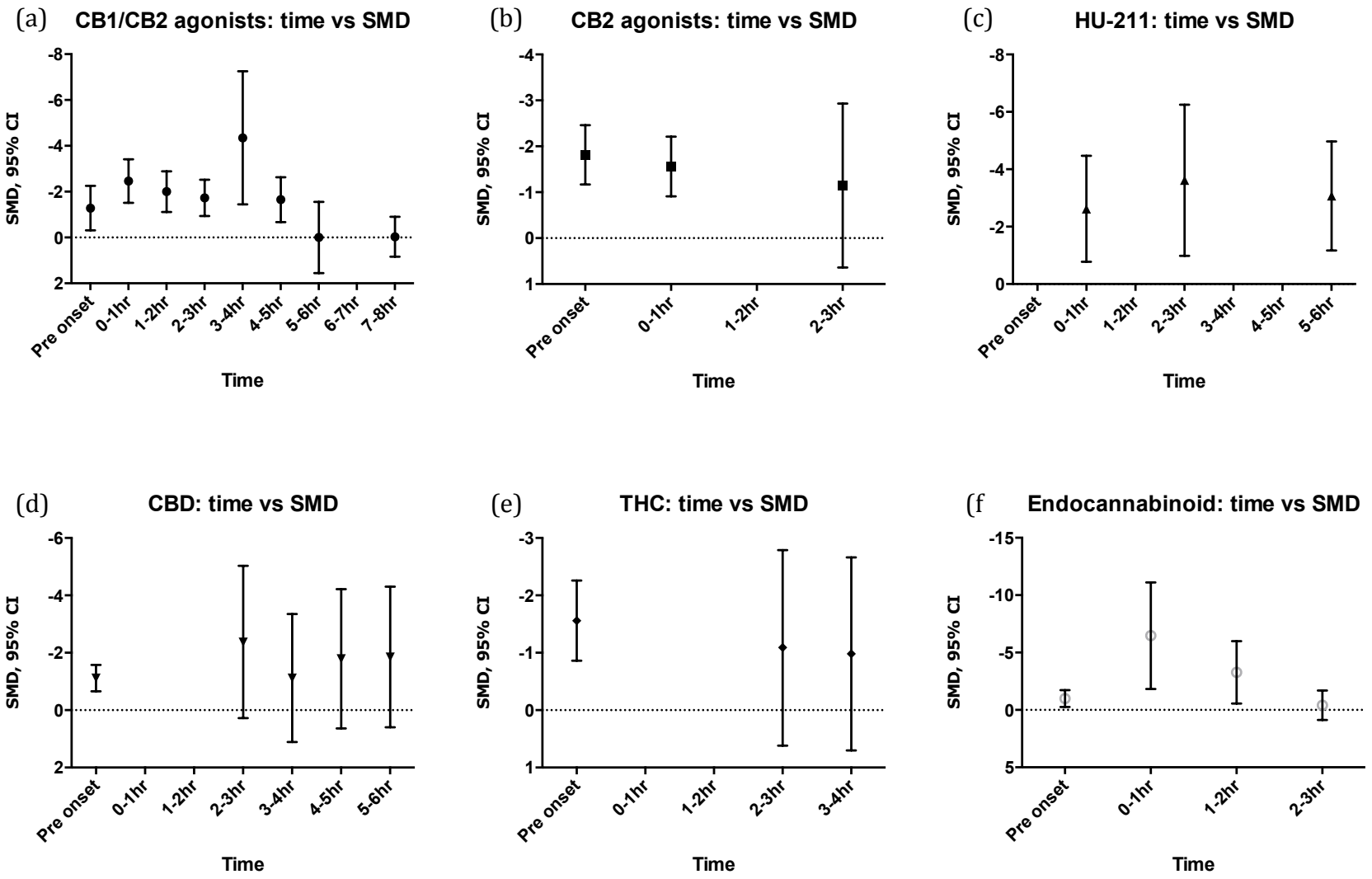
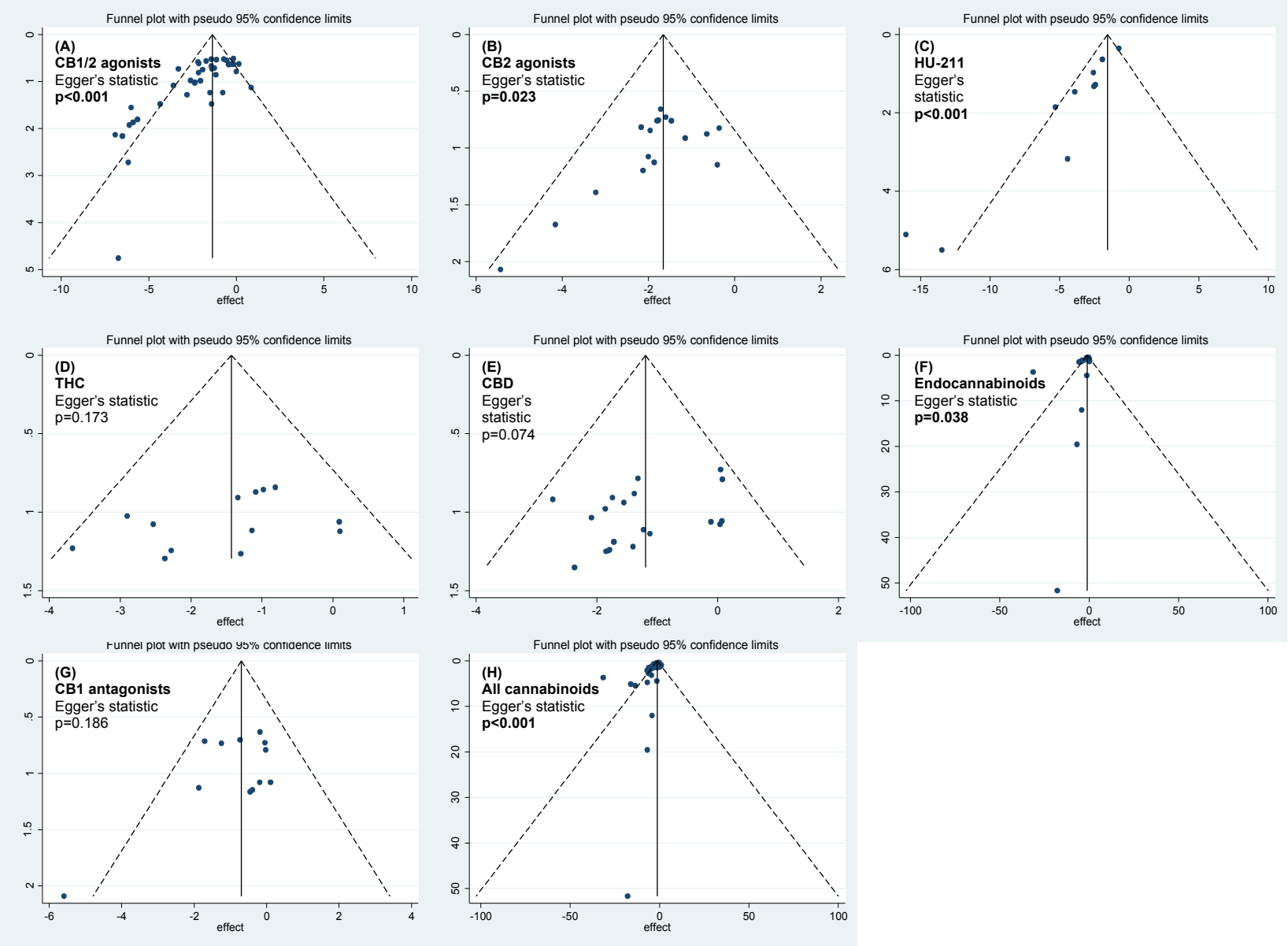

(a) CB1/CB2 agonists: time vs SMD
SMD, 95% CI
-8
-6
-4
-2
0
2
Pre onset
0-1hr
1-2hr
2-3hr
3-4hr
4-5hr
5-6hr
6-7hr
7-8hr
Time
(b) CB2 agonists: time vs SMD
SMD, 95% CI
-4
-3
-2
-1
0
1
Pre onset
0-1hr
1-2hr
2-3hr
Time
(c) HU-211: time vs SMD
SMD, 95% CI
-8
-6
-4
-2
0
Pre onset
0-1hr
1-2hr
2-3hr
3-4hr
4-5hr
5-6hr
Time
(d) CBD: time vs SMD
SMD, 95% CI
-6
-4
-2
0
2
Pre onset
0-1hr
1-2hr
2-3hr
3-4hr
4-5hr
5-6hr
Time
(e) THC: time vs SMD
SMD, 95% CI
-3
-2
-1
0
1
Pre onset
0-1hr
1-2hr
2-3hr
3-4hr
Time
(f Endocannabinoid: time vs SMD
SMD, 95% CI
-15
-10
-5
0
5
Pre onset
0-1hr
1-2hr
2-3hr
Time

Figure 5


Funnel plot with pseudo 95% confidence limits
(A)
CB1/2 agonists
Egger's statistic
p<0.001
effect
Funnel plot with pseudo 95% confidence limits
(B)
CB2 agonists
Egger's statistic
p=0.023
effect
Funnel plot with pseudo 95% confidence limits
(C)
HU-211
Egger's
statistic
p<0.001
effect
Funnel plot with pseudo 95% confidence limits
(D)
THC
Egger's statistic
p=0.173
effect
Funnel plot with pseudo 95% confidence limits
(E)
CBD
Egger's
statistic
p=0.074
effect
Funnel plot with pseudo 95% confidence limits
(F)
Endocannabinoids
Egger's statistic
p=0.038
effect
Funnel plot with pseudo 95% confidence limits
(G)
CB1 antagonists
Egger's statistic
p=0.186
effect
Funnel plot with pseudo 95% confidence limits
(H)
All cannabinoids
Egger's statistic
p<0.001
effect