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ARTICLE

## Intracerebral microdialysis of adenosine and adenosine monophosphate – a systematic review and meta-regression analysis of baseline concentrations

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Microdialysis is a method to study the extracellular space *in vivo*, based on the principle of diffusion. It can be used to measure various small molecules including the neuroregulator adenosine. Baseline levels of the compounds measured with microdialysis vary over studies. We systematically reviewed the literature to investigate the full range of reported baseline concentrations of adenosine and adenosine monophosphate in microdialysates. We performed a meta-regression analysis to study the influence of flow rate, probe membrane surface area, species, brain area and anaesthesia versus freely behaving, on the adenosine concentration. Baseline adenosine concentrations in microdialysates ranged from 0.8 to 2100 nM. There was limited evidence on baseline adenosine monophosphate concentrations in microdialysates. Across studies, we found effects of flow rate and anaesthesia versus freely behaving on dialysate adenosine concentrations ( $p \leq 0.001$ ), but not of probe membrane surface, species, or

brain area ( $p \geq 0.14$ ). With increasing flow rate, adenosine concentrations decreased. With anaesthesia, adenosine concentrations increased. The effect of other predictor variables on baseline adenosine concentrations, for example, post-surgical recovery time, could not be analysed because of a lack of reported data. This study shows that meta-regression can be used as an alternative to new animal experiments to answer research questions in the field of neurochemistry. However, current levels of reporting of primary studies are insufficient to reach the full potential of this approach; 63 out of 133 studies could not be included in the analysis because of insufficient reporting, and several potentially relevant factors had to be excluded from the analyses. The level of reporting of experimental detail needs to improve.

**Keywords:** adenosine, meta-analysis, microdialysis, systematic literature review.

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Microdialysis is a simple and elegant method to study biological processes in the extracellular space *in vivo*. It is based on the principle of diffusion; molecules diffuse through a semi-permeable membrane into a continuously

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*Abbreviations used:* AMP, adenosine monophosphate; ANOVA, analysis of variance; ATP, adenosine triphosphate; DNA, deoxyribonucleic acid; EROS, Early Review Organizing Software; HPLC, high pressure liquid chromatography; RNA, ribonucleic acid; SD, standard deviation; SEM, standard error of the mean; SR, systematic literature review.

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flowing isotonic fluid. Sampling at regular intervals (minutes) allows the investigator to monitor concentrations of the molecule of interest over time (hours–days). Microdialysis can be used to measure various small molecules including neurotransmitters. Besides by biological variation, concentrations of these molecules in the dialysate are affected by, for example, flow rate, membrane type and membrane surface area (Westerink and Cremers 2007). Concentrations therefore can vary substantially between laboratories, but also between individual animals within groups.

Adenosine is a purine nucleoside that is a building block for deoxyribonucleic acid and ribonucleic acid. As a main metabolite of adenosine triphosphate, it is also involved in the cellular energy metabolism. Besides, it is a neuroregulator with specific receptors in the brain, which can be antagonized by caffeine. Many studies have shown that adenosine is involved in the sleep–wake regulation (e.g. Basheer *et al.* 1999; Blanco-Centurion *et al.* 2006; Sharma *et al.* 2010b; Vazquez-DeRose *et al.* 2014). The first report of adenosine being analysed by microdialysis appeared in 1982 (Zetterstrom *et al.* 1982). So far, an overview of the variation in baseline dialysate concentrations of adenosine is lacking. Such an overview could improve comparisons between laboratories. This could benefit the design of future primary studies.

Systematic literature review (SRs) are common practice in clinical research and are considered to provide the highest level of evidence (Hooijmans *et al.* 2010a). Most clinical SRs determine the effectiveness of an intervention. In animal research, SRs are still less common, and most animal SRs focus on specific interventions or descriptions of the available animal models. While SRs of research techniques used in animal studies are even less common, an SR is the optimal method to acquire a complete overview of the past use of a technique.

SRs differ from narrative reviews by using a thorough transparent methodology to retrieve, select and analyse all relevant papers for the review (de Vries *et al.* 2014b). While many interesting narrative reviews on microdialysis are available (refer to the microdialysis handbook by Westerink and Cremers (2007) for an excellent selection), only few SRs of the microdialysis technique have been performed to date. Examples comprise cocaine-induced dopamine increases in the nucleus accumbens (Frank *et al.* 2008); ethanol-induced alterations in glutamate and GABA (Fliegel *et al.* 2013) and monoamines (Brand *et al.* 2013); and serotonin neurotransmission after selective serotonin reuptake inhibitors (Fritze *et al.* 2017).

To provide evidence at an integrated level on the observed range of baseline adenosine levels in microdialysates, we performed an SR. The nucleotide adenosine monophosphate (AMP) can be measured simultaneously with adenosine because of close chemical resemblance (Ballarin *et al.* 1989). Because we expected many studies to report both

compounds, we extended our review to include AMP concentrations.

Microdialysis reflects changes in the extracellular concentrations of the analysed molecules, but does not inform us of their exact concentrations, because the recovery of the compound of interest is < 100% (Westerink and Cremers (2007)). In this SR, we do not aim to estimate the actual *in vivo* concentrations. We restrict ourselves to providing a complete overview of concentrations in brain dialysates. Besides, to study the influence of potential explanations for differences in baseline concentrations, such as flow rate, probe membrane surface, species, brain area and anaesthesia versus freely behaving, we conducted a meta-regression analysis.

## Materials and methods

On 25 January 2016, we registered the protocol (Van der Mierden *et al.* 2016) for an SR addressing the research question: ‘What is the reported range of concentrations of adenosine and AMP in intracerebral microdialysates?’. The protocol describes the used methodology for this SR and is compliant with the Transparency and Openness Promotion (TOP) guidelines. It is available on the protocol section of the SYRCLE website ([www.SYRCLE.nl](http://www.SYRCLE.nl)).

### Search and selection

We developed an extensive search strategy for finding all relevant animal studies on adenosine or AMP using microdialysis for three databases: PubMed, EmBase and Web of Science. The search strategies are described in our protocol (Van der Mierden *et al.* 2016). For PubMed and EmBase, we used the previously published filters for animal studies (Hooijmans *et al.* 2010b; de Vries *et al.* 2014a). For Web of Science no animal filter was used, as no validated animal filter is available. Searches were performed on 25 January 2016. Duplicates and triplicates were removed manually. Reference lists of included papers were checked for additional papers.

Screening of titles and abstracts was performed in Early Review Organising Software (Institute of Clinical Effectiveness and Health Policy, Buenos Aires, Argentina) by two independent reviewers (SvdM and CHCL). Screening of full-text papers was performed by three independent reviewers (SvdM, SAS and CHCL); each paper was checked for eligibility by two of these three reviewers. Discrepancies were resolved by discussion among the reviewers. We excluded studies on other techniques than microdialysis (e.g. biosensors and studies using precursors to microdialysis such as ventricular, cortical cup and push-pull perfusion), studies measuring other substances than adenosine or AMP (e.g. cyclic-AMP (Mork and Geisler 1994) and adenosine metabolites (Salem and Hope 1999), extra-cerebral microdialysis studies, human and *in vitro* studies and (in the full-text screening phase only) papers not containing primary study data. We also excluded retro-dialysis studies that did not report baseline values without adenosine in the perfusion fluid. We included primary studies measuring baseline AMP and/or adenosine by microdialysis in animals other than humans, also when they did not report their baseline data.

### Study characteristics and quality assessment

We extracted data on the animal model (e.g. species and sex), the technique (microdialysis; e.g. flow rate and probe length) and two outcome measures: baseline concentration of adenosine and AMP in microdialysate. Study characteristics were tabulated (Appendices S1–S3). The full list of extracted study characteristics is provided in our protocol (Van der Mierden *et al.* 2016). To obtain an indication of the average reporting quality of the included studies, percentages of reported study characteristics were determined per paper.

### Data analysis

In this paper, concentrations are given in nM. We converted concentrations to nM where necessary. When papers reported correction for recovery, we converted the concentrations back to the concentrations in dialysate. If concentration data were only available graphically, Universal Desktop Ruler (AVPSoft, <https://avpsoft.com/products/udruler/>) was used to estimate the values. Probable typographical errors in the dose of the anaesthetic (Berman *et al.* 2000) and the flow rate (McKenna *et al.* 2007) were corrected. Probable typographical errors in Nomoto *et al.* (1999) and Nomoto *et al.* (2000) could not be corrected, and these data were excluded from Table 1 and subsequent analyses. Data from (Masana *et al.* 1989) and (Tominaga *et al.* 1992) were extracted as far as possible without understanding Japanese. All other included papers were in English. Of the corrected papers, only the one by Berman *et al.* (2000) was included in the meta-regression. We did not perform a sensitivity analysis excluding these data.

When papers reported several subsequent baseline concentrations with the corresponding standard error of the mean (SEM) or standard deviation (SD), the last baseline measurement (i.e. before starting treatment) with the corresponding SEM was included in Table 1. If only a single mean value or a baseline range was presented, this was included in Table 1. When a range of adenosine concentrations was given, we report the lower and upper boundary of the range. For papers describing several separate experimental groups, the groups were treated as independent experiments (indicated by capital letters in the study-ID), unless only overall average baseline concentrations were provided.

In microdialysis studies, several brain regions can be measured simultaneously within the same animal. When the same brain region was sampled on both sides of the brain, an average adenosine concentration and the corresponding SEM was calculated; we conservatively assumed a correlation of one between both sides, and calculated the mathematical average. (Borenstein *et al.* 2009). When different brain regions were measured within the same animal, we treated the brain region as the experimental unit (instead of the animal). That is, if adenosine was measured in multiple brain regions (partially) within the same animals, values are reported for all brain regions separately, and included in the meta-regression analysis. Six papers reported data from two brain regions that were (partially) measured within the same animals.

To determine if the sample of studies included in our meta-regression was representative of the full sample of included studies, baseline adenosine levels were compared between included and excluded studies with the non-parametric independent samples Mann–Whitney *U* test, using SPSS (IBM Corp. IBM SPSS Statistics for Windows. Armonk, NY, USA) Boxplots were also created in SPSS.

**Table 1** Baseline adenosine in dialysates from lowest to highest concentration

Study reference	Adenosine (nM)	SEM (nM)
Masana <i>et al.</i> (1989)	0.8	0.1
Savelyev <i>et al.</i> (2012)	1.28	0.08
Kalinchuk <i>et al.</i> (2011)A	2.3	0.7
Wigren <i>et al.</i> (2007)	4.3	0.4
Nagel and Hauber (2004)	4.8	0.38
Kalinchuk <i>et al.</i> (2011)B	6	2
Okada <i>et al.</i> (2003)	6	1.7
Carrozzo <i>et al.</i> (2012)	7	7
Lehmann <i>et al.</i> (1987)	10	9
Van Wylen <i>et al.</i> (1986)	10	
Nagel and Hauber (2002)	10.51	2.61
Melani <i>et al.</i> (2012)A	11.72	0.2
Ballarin <i>et al.</i> (1991)A	12	0.9
Basheer <i>et al.</i> (1999)	12.1	2.3
Melani <i>et al.</i> (1999a)	13	1
Fu <i>et al.</i> (2015)	13.1	1.5
Melani <i>et al.</i> (2012)B	13.15	0.4
Ballarin <i>et al.</i> (1991)B	14	2
Gianfriddo <i>et al.</i> (2003)	14	1
Pazzagli <i>et al.</i> (1995)A	15.5	0.2
Matsumoto <i>et al.</i> (1992)A	16	
Pazzagli <i>et al.</i> (1995)B	16.6	0.2
Pinna <i>et al.</i> (2002)	17	2
Song <i>et al.</i> (2012)A	18	3
Melani <i>et al.</i> (2003)	19	2
Bennett <i>et al.</i> (2003)A	20.3	3.76
Nelson <i>et al.</i> (2009)A	20.9	2.5
Gianfriddo <i>et al.</i> (2004)A	23	2
Melani <i>et al.</i> (1999b)	25	2
Bennett <i>et al.</i> (2000)	25–100	
Sharma <i>et al.</i> (2010a)	26.9	9.4
Song <i>et al.</i> (2012)B	28	5
Lutz and Kabler (1997)	29–73	4–9
Lydic <i>et al.</i> (2010)	29.9	4.4
Butcher <i>et al.</i> (1987)	30	10
Porkka-Heiskanen <i>et al.</i> (1997)	30	9.5
Maysinger <i>et al.</i> (1992)B	31	21
Gianfriddo <i>et al.</i> (2004)B	32	3
Porkka-Heiskanen <i>et al.</i> (2000)	32.8	3
Matsumoto <i>et al.</i> (1992)B	33	
Pazzagli <i>et al.</i> (1994)	34.9	3 (?)
Bennett <i>et al.</i> (2003)B	39	7
Mijangos-Moreno <i>et al.</i> (2014)B	39	4
Moss <i>et al.</i> (1995)	40–190	
Nelson <i>et al.</i> (2009)B	42	6.3
Andine <i>et al.</i> (1990)	47	
Hagberg <i>et al.</i> (1987)	49.9	9.1 (?)
Dux <i>et al.</i> (1990)A	51	6
Scheller <i>et al.</i> (1996)	52	6
Aden <i>et al.</i> (2004)	53	14
Grabb <i>et al.</i> (1998)D	60	10
Herrera-Marschitz <i>et al.</i> (1994)A	60	18 (?)

(continued)

Table 1. (continued)

Study reference	Adenosine (nM)	SEM (nM)
Koos <i>et al.</i> (1997)	65	12
Miranda <i>et al.</i> (2014)	67	3
Murillo-Rodriguez <i>et al.</i> (2004)B	67	1
Kjellmer <i>et al.</i> (1989)B	68	7
Perez-Pinzon <i>et al.</i> (1993)	68	29
Chen <i>et al.</i> (1993)	70	
Herrera-Marschitz <i>et al.</i> (1994)B	70	15 (?)
Murillo-Rodriguez <i>et al.</i> (2008)	70	4.22
Berman <i>et al.</i> (2000)B	69.7	9.8
Berman <i>et al.</i> (2000)C	69.8	5.2
Zhu <i>et al.</i> (2001)B	71.7	84.9
Dux <i>et al.</i> (1990)B	72.8	11
Park <i>et al.</i> (1988)	80	
Berman <i>et al.</i> (2000)D	81.7	14.6
Kaku <i>et al.</i> (2001)	82.3	3
Peigen and Jing (1997)A	83	9
Peigen and Jing (1997)B	84	6.9
Peigen and Jing (1997)C	87	7.3
Deng <i>et al.</i> (2003)	87–360	
Morimoto <i>et al.</i> (1991)	90	15
Skarphedinsson <i>et al.</i> (1989)	90	
Chen <i>et al.</i> (1992)	100	10
Hillered <i>et al.</i> (1989)	100	0
Kaku <i>et al.</i> (1994)A	100	20
Kaku <i>et al.</i> (1994)B	100	20
Dobolyi <i>et al.</i> (1997)	100–300	
Berman <i>et al.</i> (2000)A	106.1	25.9
Kjellmer <i>et al.</i> (1989)A	107	19
Maysinger <i>et al.</i> (1992)A	107	23
Chen and Stone (1991)	110	10
Ruth <i>et al.</i> (1993)	110	5.8
Schulte <i>et al.</i> (2004)	110	45
Murillo-Rodriguez <i>et al.</i> (2004)A	112	2
Mijangos-Moreno <i>et al.</i> (2014)A	120	2
Mijangos-Moreno <i>et al.</i> (2015)	120	3
Watson <i>et al.</i> (1999)	120	17.4
Valtysson <i>et al.</i> (1998)	130	
Britton <i>et al.</i> (1999)A	140	
Ballarin <i>et al.</i> (1989)	144	60
Kim <i>et al.</i> (2010)	165	19.7
Carswell <i>et al.</i> (1997)	184	10.5
Britton <i>et al.</i> (1999)B	190	
Yan <i>et al.</i> (1995)	190	40
Ballarin <i>et al.</i> (1987)	200	30
Giddy <i>et al.</i> (1996)	200	28
Grabb <i>et al.</i> (1998)A	200	
Headrick <i>et al.</i> (1994)	200	
Cui <i>et al.</i> (2013)	204	18.5
Blanco-Centurion <i>et al.</i> (2006)	216	57
Cui <i>et al.</i> (2016)	230	9.5
Pazzagli <i>et al.</i> (1993)A	240	30 (?)
Dobolyi <i>et al.</i> (1998)	285	8
Grabb <i>et al.</i> (1998)B	300	100

(continued)

Table 1. (continued)

Study reference	Adenosine (nM)	SEM (nM)
Li <i>et al.</i> (2011)A	310	
Li <i>et al.</i> (2011)B	320	
Pazzagli <i>et al.</i> (1993)B	330	20 (?)
Zhang and Niu (1994)	330	50
Zetterstrom <i>et al.</i> (1982)	340	
Kondoh <i>et al.</i> (1999)	390	90
Richter <i>et al.</i> (1999)	390	14.4
Grabb <i>et al.</i> (1998)C	400	
Sciotti and Van Wylen (1993a)	420	30
Dohmen <i>et al.</i> (2001)A	480	
Dohmen <i>et al.</i> (2001)B	480	
Li <i>et al.</i> (2011)C	480	70
Materi and Semba (2001)	480	
Sciotti and Van Wylen (1993b)A	530	70
Sciotti and Van Wylen (1993b)B	570	69.3
Sciotti <i>et al.</i> (1992)	600	100
Zhu <i>et al.</i> (2001)A	619.9	212.2
Northington <i>et al.</i> (1992)	636.5	53.6
Dobolyi <i>et al.</i> (1999)A	650	140
Nilsson <i>et al.</i> (1990)A	730	
Nilsson <i>et al.</i> (1990)B	730	
Nilsson <i>et al.</i> (1990)C	730	
Dobolyi <i>et al.</i> (1999)B	740	100
Tominaga <i>et al.</i> (1992) In Japanese	800	160 (?)
Slezia <i>et al.</i> (2004)	950	210
Dobolyi <i>et al.</i> (2000)	970	40
Sciotti <i>et al.</i> (1993)A	1600	
Sciotti <i>et al.</i> (1993)B	2100	350

Capital letters after the year of publication within the literature reference indicate separate subgroups within the same paper. Lower case letters indicate separate papers. Question marks indicate uncertainty on the reported value being an SEM or an SD.

#### Meta-regression

While writing the protocol, we did not anticipate sufficient data to be available for a meta-regression. Therefore, the analysis described below was designed after finishing the data-extraction, and thus was not pre-specified. Meta-regression analysis was performed in R version 3.4.2 (R\_Core\_Team 2016) with the metafor package (Viechtbauer 2010), using the *rma* function (*knha* = TRUE). We used the method suggested by Higgins *et al.* to transform the reported average adenosine concentrations and corresponding SDs to account for the skew of the data (Higgins *et al.* 2008).

The following moderator variables were included: probe surface area (estimated by probe length  $\times \pi \times$  probe diameter), flow rate, species, brain area and anaesthesia versus freely behaving. The analysis of variance function was used to test for overall effects of categorical moderators. Because several brain regions had only been measured in a single study, different brain areas were pooled for the analyses: the basal ganglia were pooled with the basal forebrain; nuclei in the brainstem were pooled as such; thalamic and subthalamic nuclei were pooled as thalamus; and all cortical regions were pooled as cortex. As we could not include post-surgical

recovery period in our analyses, we show the baseline adenosine concentrations by recovery period in a boxplot. Because dialysis experiments mostly start either after 24 h or after 1 week of recovery, we plotted studies with recovery periods  $\leq 24$  h next to those with periods  $> 24$  h.

A sensitivity analysis was performed in which we replaced SDs with a value below 4 by the value 4 (nM).

## Results

### Search and selection

Our search in PubMed retrieved 539 references, that in Embase 616 and that in Web of Science 758. After removal of duplicates, 981 references remained for title-abstract screening. Of these, 196 were screened full-text, and 128 were included in this review. From the reference lists of the included papers, we identified another four papers on intracerebral microdialysis of adenosine. An overview of the flow of papers is provided in Fig. 1.

### Study characteristics and quality assessment

Our search retrieved 132 papers describing intracerebral microdialysis of adenosine or AMP, their characteristics are listed in Appendix S1 (those describing an actual baseline

adenosine concentration in dialysate), Appendix S2 (those describing adenosine microdialysis without a baseline concentration) and Appendix S3 (those describing an actual baseline AMP concentration in dialysate). The reporting quality of the included studies is expressed by the percentages of papers reporting specific items.

Of the 132 papers, 110 (83%) reported the number of animals used, which ranged from 3 to 58 per paper. Ninety-seven papers (73%) were on rats; 43 on Sprague-Dawleys, 37 on Wistars, 2 on F344s, 1 on Albino Sprague-Dawleys, 1 on Long-Evans and 12 left the strain unspecified. Mice were used in 9 papers (7%); C57BL/6 in 5, other strains in 3 and 1 paper left the strain unspecified. The remaining papers were on cats (8), pigs (7), sheep (4), gerbils (3), marmosets (2), rabbits (1) and turtles (1).

The sex of the animals used was reported in 99 papers (75%); 92 were in male animals only, 1 in female animals only and 6 in both sexes. The authors of 55 papers (42%) provided some information on the animal housing, mostly just the temperature and humidity. The light-dark cycle was reported in 45 papers (34%), but only 25 (19%) provided the actual times. For dialysis experiments in freely behaving animals, post-surgical recovery ranged from 16 h to  $> 3$  weeks. The post-surgical recovery period was described by 44 papers (33%); 2 only mentioned 'later'.

Recovery rate of the probe was described to some extent in 46 papers (35%), and ranged from 2.6% to 47.8%. Sampling bin time was reported by 108 papers (82%), and lasted from 1 to 60 min. Information on the analysis method of adenosine was provided by 124 papers (94%); 120 used some form of high pressure liquid chromatography (HPLC), one used mass spectrometry, one used capillary electrophoresis and one used a radioimmunoassay. Total dialysis experiment duration could be retrieved from 102 papers (77%) and ranged from 30 min to  $> 54$  h.

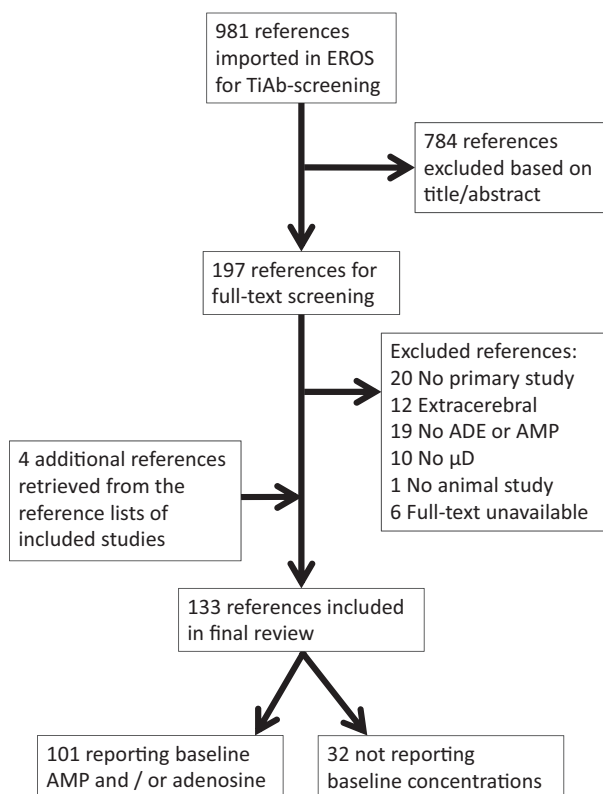
### Adenosine data

Our search retrieved 98 papers reporting actual baseline adenosine concentrations in dialysate for one or more experimental groups. Absolute adenosine concentrations in brain dialysates, ranging from 0.8 to 2100 nM, were reported for 133 experimental animal groups in the 98 papers, and are listed in Table 1.

Of the 98 papers, 63 papers reported the SEM and 16 the SD. There were four papers reporting SEM or SD without indicating which measure was used, and for one paper (in Japanese), we were not sure, all indicated by a question mark in Table 1.

### AMP data

Of the papers retrieved by our search, only two presented the absolute AMP concentrations in brain dialysate, for three experimental groups in total. Brain dialysate AMP



**Fig. 1** Flow scheme of retrieved and included references. Note that only two papers report baseline adenosine monophosphate concentrations.

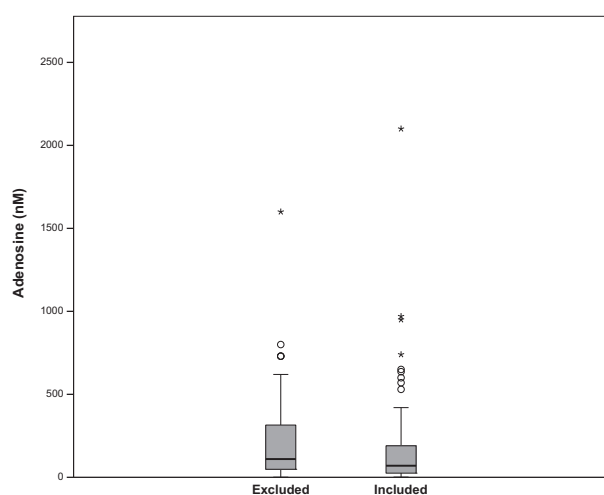
concentration ranged from 14 to 940 nM. Details on these studies are provided in Appendix S3.

### Meta-regression of the effect of experimental settings on dialysate adenosine concentrations

#### Included studies

The 74 studies reporting the moderators probe surface area (i.e. probe length and diameter), flow rate, species, brain area and anaesthesia versus freely behaving, as well as the baseline adenosine concentration in dialysate and the corresponding SEM or SD (all listed in Table 1), were included in the meta-regression. A box-plot of the adenosine concentrations for included and excluded studies is provided in Fig. 2. The reported adenosine concentrations were not significantly different between included ( $k = 74$ ) and excluded ( $k = 59$ ) studies ( $p = 0.099$ ).

Of the 74 studies included in the meta-regression, 25 were in freely behaving animals, 49 in anaesthetised animals. For the 25 studies in freely behaving animals, post-surgical recovery time was reported in 20 (range: 24 h–3 weeks). For the 49 studies in anaesthetized animals, post-surgical recovery time was only reported in 2 (both 24 h). Post-surgical recovery time could thus not be analysed in our meta-regression analysis. To provide an indication of the effect of post-surgical recovery duration on the baseline adenosine concentrations, we plotted them for the 20 studies in freely behaving animals reporting post-surgical recovery time in



**Fig. 2** Adenosine concentrations (nM) of studies included and excluded in the meta-regression. The horizontal bar in the boxplot reflects the median value, the box the 25th and 75th percentile, the whiskers the range excluding outliers, the circles outliers (outside  $1.5\times$  the interquartile range) and asterisks extreme outliers (outside  $3\times$  the interquartile range). The reported adenosine concentrations were not significantly different between included ( $k = 74$ ) and excluded ( $k = 60$ ) experimental animal groups ( $p = 0.099$ ).

Fig. 3. No statistical analyses were performed because of the low numbers of studies.

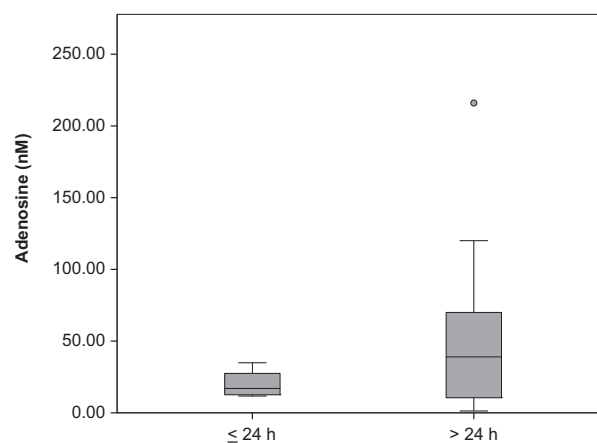
#### Meta-regression of adenosine concentrations in dialysate

The meta-regression included five moderators; two numerical and three categorical. The observed values of these moderators are presented in Table 2. Only anaesthesia and flow rate significantly affected dialysate adenosine concentrations (Table 2). These results were confirmed by the sensitivity analysis. Heterogeneity was high;  $I^2 = 99.73\%$ .

Figure 4 shows a bubble plot of predicted and actual adenosine concentrations by flow rate, reflecting the overall decrease in concentration with increasing flow rate. Figure 5 shows a plot of the predicted and actual adenosine concentrations by type of anaesthesia, reflecting an increase in adenosine with all types of anaesthesia compared to non-anaesthetised (i.e. freely behaving).

### Discussion

This SR shows that the reported baseline concentrations of adenosine in microdialysates from laboratory animals cover a wide range; from 0.8 to 2100 nM. We expected the baseline values to cover a smaller range. To the best of our knowledge, no overview of baseline adenosine concentrations has been published before. The range of concentrations observed in our SR seems to be larger than the range described in, for example, a narrative review of acetylcholine (Pepeu and Giovannini 2007) and a SR of noradrenaline, serotonin and dopamine (Brand *et al.* 2013), both restricted



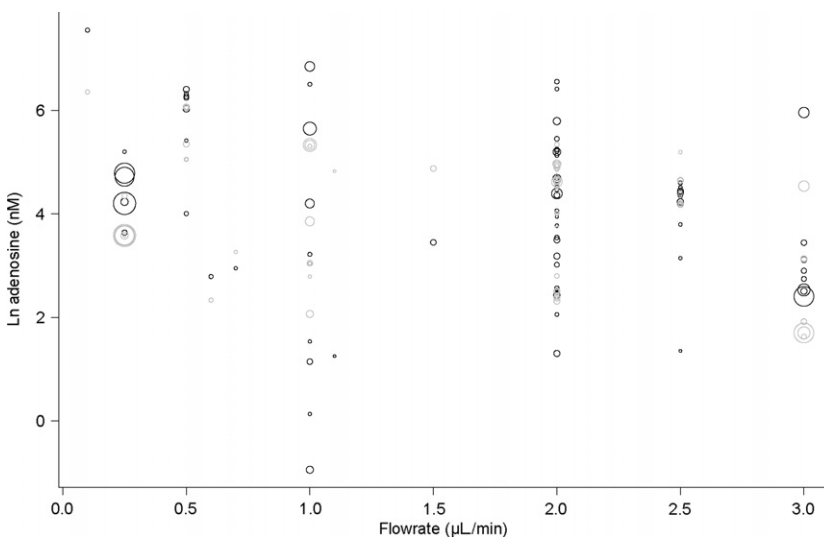
**Fig. 3** Adenosine concentrations (nM) of studies in freely behaving animals by duration of post-surgical recovery ( $\leq 24$  h vs.  $> 24$  h). The horizontal bar in the boxplot reflects the median value, the box the 25th and 75th percentile, the whiskers the range excluding outliers and the circle an outlier (outside  $1.5\times$  the interquartile range).  $k = 7$  for experimental animal groups with post-surgical recovery  $\leq 24$  h and  $k = 13$  for those with recovery  $> 24$  h. No statistical analyses were performed because of the low numbers of studies.

**Table 2** Moderators in the meta-regression

Moderator	Range or values	Estimate from MR (SE)	<i>p</i> -Value from MR
Flow rate (N)	0.1–3 $\mu\text{L}/\text{min}$	−0.74 (0.19)	< 0.001
Probe surface area (N)	0.24–2.84 $\text{mm}^2$	0.13 (0.32)	0.681
Anaesthesia (C)	None, injection, inhalation, combined (injection & inhalation)		< 0.001
	Injection versus none	2.51 (0.44)	< 0.001
	Inhalation versus none	1.50 (0.43)	0.008
	Combined versus none	2.13 (0.78)	0.008
Species (C)	Cat, mouse, pig, rat, sheep		0.672
	Cats versus rats	1.37 (1.02)	0.186
	Mice versus rats	0.35 (0.84)	0.677
	Pigs versus rats	0.74 (0.90)	0.414
	Sheep versus rats	0.18 (0.91)	0.840
Brain region (C)	Basal forebrain, brainstem, cortex, hippocampus, thalamus <sup>a</sup>		0.145
	Brainstem versus basal forebrain <sup>a</sup>	−0.98 (0.68)	0.153
	Cortex versus basal forebrain <sup>a</sup>	−0.26 (0.62)	0.682
	Hippocampus versus basal forebrain <sup>a</sup>	0.74 (0.50)	0.145
	Thalamus versus basal forebrain <sup>a</sup>	0.76 (0.66)	0.253

N, numerical moderator; C, categorical moderator; MR, meta-regression.

<sup>a</sup>Refer to the methods section for information on pooling of brain regions.

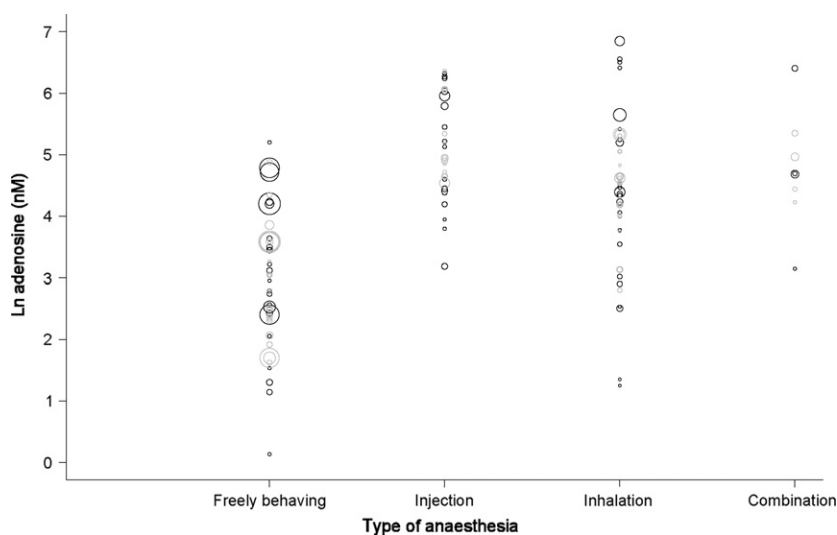


**Fig. 4** Predicted and actual adenosine concentration by flow rate. Black bubbles reflect actual values, grey bubbles values predicted by the regression model. Bubble size reflects precision (inverse variance) of the studies. Statistical details are provided in the text and Table 2.

to a subset of studies with more comparable experimental designs. Most reviews on microdialysis do not describe baseline levels.

Although we did include thesaurus terms for AMP in our searches (MEDical Subject Heading ‘AMP’ in PubMed and ‘adenosine phosphate’ in Embase), besides searching for it in the title, abstract and keywords, we only found two papers reporting absolute AMP concentrations in brain dialysates. We expect that more authors have measured AMP in dialysate, but possibly it was not reported as the main compound of interest. For future SRs on AMP, we recommend extensive *a priori* scoping searches and adaptation of the search strategy.

While analysing the data for this review, we came across a few older synonyms for the term ‘microdialysis’ that we did not include in our search. In future searches for microdialysis studies, we recommend including the terms ‘chemitrode’, ‘dialyetrode’, ‘brain dialysis’, ‘intracerebral dialysis’, ‘intracranial dialysis’, ‘transcranial dialysis’ and ‘implanted perfused hollow fibre’. From the four papers that were retrieved from the reference lists, three (Zetterstrom *et al.* 1982; Van Wylen *et al.* 1986; Park *et al.* 1988) would have been found by searches in PubMed, Embase and Web of Science, had these terms been included. From the same four papers, only one conference abstract (Chen and Stone 1991) is not indexed in any of these databases.



**Fig. 5** Predicted and actual adenosine concentration by type of anaesthesia. Black bubbles reflect actual values, grey bubbles values predicted by the regression model. Bubble size reflects precision (inverse variance) of the studies. Statistical details are provided in the text and Table 2.

Compared to other substances, microdialysis of adenosine has the added challenge of its source not being purely neurogenic. As a building block for deoxyribonucleic acid and ribonucleic acid and the main metabolite of adenosine triphosphate, the adenosine measured in the dialysates could originate from metabolic processes (Melani *et al.* 2014) independent of neurotransmission. Our meta-regression shows that anaesthesia and flow rate affect the baseline dialysate adenosine concentrations. Our finding that adenosine concentrations decrease with increasing flow rate are in line with the basic concept of diffusion (i.e. with a higher flow rate there is less time for analytes to diffuse over the membrane, resulting in lower concentrations) and with experimental findings on other substances (e.g. Rooyackers *et al.* 2013 and reviewed by Fliegel *et al.* 2013).

Preceding experimental work shows the effect of anaesthesia on the extracellular levels of various neurotransmitters (reviewed in de Souza Silva *et al.* 2007). In line with these findings, all types of anaesthesia increase the concentration of adenosine according to our meta-regression results. While microdialysis studies on adenosine have been performed since 1982, microdialysis of freely behaving animals was uncommon in the early stages. Most of the included studies using anaesthesia were published before the year 2000, most of the studies in freely behaving animals afterwards (refer to Appendix S1). Methodology generally improves over time, which could result in increased recovery; newer studies could find higher baseline concentrations. We thus cannot exclude the effect of anaesthesia on baseline adenosine levels being confounded to some extent by year of publication. The actual effect may thus be larger than the observed effect.

In addition, studies measuring under anaesthesia may well start immediately after surgery, while studies in freely behaving animals will usually implement a recovery period before the onset of measurements. The interval between surgery can affect the adenosine concentrations; adenosine

decreases substantially over time since surgery (Pazzagli *et al.* 1993). This decrease could become even more pronounced with the development of gliosis over the course of several days after surgery. Figure 3 shows, however, that for the included sample of studies, increased lag times result in more variation between the measured adenosine values, but not in clearly decreased concentrations. For our analysis, this limits the confounding by post-surgical lag time for freely behaving animals. Of note, analyses of the effects of anaesthesia are further complicated by other surgery-related factors, such as stress, and variables that are inherently associated with anaesthesia, such as the lack of movement, that could potentially explain part of the observed increases in adenosine concentrations.

Based on tissue sections and HPLC with electrochemical detection, baseline levels of purines have been shown to vary significantly between brain regions as well as between different mouse strains (Pani *et al.* 2014). We tabulated the brain regions as mentioned by the authors, and collated these into the wide categories for the meta-regression. While this results in the loss of information, the number of studies for most brain regions is still rather low for the analyses. At this stage, the effect of brain region on baseline dialysate adenosine concentration remains unclear.

Different types of chromatography with ultraviolet or fluorescence detection are the most common method for determination of nucleotides and nucleosides, including AMP and adenosine (Khlyntseva *et al.* 2009). One hundred and twenty-one of the papers included in this review used some form of HPLC. The advantage of HPLC is that it allows for a rapid determination of several substances simultaneously. The disadvantage of fluorescent detection is the necessity of converting substances into the fluorescent form (by means of derivatisation with chloroacetaldehyde), which may lead to hydrolysis of nucleotides and, thus, to loss of substance during sample preparation. While the reporting



of the basic type of analysis method in microdialysis papers is relatively high, the description of the analysis methods can be rather minimal. As the analysis methods compare dialysate concentrations to a given standard, we do not expect major effects and did not include them as a moderator in the meta-regression.

We did not pre-specify our meta-regression in our registered protocol, because we did not anticipate sufficient data to be available. Even though the analysis was designed and conducted post hoc, and even though we included only those moderators for which sufficient data were available, we do not expect it to have biased our conclusions.

Microdialysis baseline concentrations depend strongly on, for example, exact probe location and arousal level of the animal. Because of this, many authors only report effects as percentages of baseline, without reporting baseline levels, resulting in exclusion of 33 papers from our meta-regression. The low levels of reporting of experimental details resulted in another 63 being excluded from the meta-regression.

The low levels of reporting of experimental details also limited the moderators that could be included in the analyses. Before data-extraction, we considered including the following additional moderators: membrane type or permeability, sterilisation procedure, sample storage before analysis, washout time, post-operational recovery time and clock-time of measurements. These factors are known to affect dialysate concentrations of certain compounds (Westerink and Cremers 2007), by physiological mechanisms, by degradation of adenosine over time or by variations in recovery because of, for example, glial cells around the probe. These factors were, however, reported too infrequently to analyse.

We did not perform a risk of bias assessment for this SR, as we analyse baseline concentrations, completely disregarding any subsequent intervention. Most risk of bias-tools (e.g. Hooijmans *et al.* 2014) focus strongly on interventions and presuppose a comparison between an experimental group and control group; these are therefore not relevant to our research question. We did provide an indication of average study quality by calculating the percentages of studies reporting relevant study characteristics.

Publication bias can result from a relative surplus in the literature of studies showing positive effects of an intervention. We did not formally analyse publication bias as it seems hardly relevant when addressing baseline concentrations. For microdialysis studies, baseline concentrations are not always reported; many authors choose to report percentages of baseline only, and this could be more common when finding extremely high or low baseline values. We expect the reporting of extreme baseline concentrations, however, to be equally affected on both sides of the distribution of dialysate adenosine concentrations.

Our meta-regression is a proof of principle for quantification of the effects of experimental methodology (in microdialysis experiments) on the outcome measure (adenosine

concentration in microdialysate) at the meta-level, with data from literature, without using new animals. This is one of the few SRs and meta-analyses characterizing microdialysis as an experimental method in laboratory animal studies. To the best of our knowledge, this paper describes the first SR of microdialysis of adenosine.

Other SRs of the microdialysis technique include, for example (Brand *et al.* 2013), whose fixed-effect meta-analysis showed that baseline microdialysate concentrations of noradrenaline, serotonin and dopamine hardly vary by sex, strain, brain region or state of consciousness, but that they increase throughout the brain after ethanol. Besides, a meta-analysis by (Frank *et al.* 2008) indicated that cocaine-induced dopamine overflow depends on dose, brain region and on route of administration, but not on species (rats vs. mice), strain, age, sex, or calcium concentration of the perfusion medium. Furthermore, according to the meta-analyses by Fliegel *et al.* (2013), dialysate glutamate and GABA concentrations change with exposure to and withdrawal from ethanol. Moreover, meta-regression has shown a relationship between serotonin and duration of selective serotonin reuptake inhibitor treatment in the frontal cortex in rats (Fritze *et al.* 2017). Combined, these results show the power of meta-analysis as an alternative to performing new animal experiments in answering various research questions in the field of neurochemistry.

We here provide a complete overview of the baseline adenosine concentrations in microdialysis studies, showing a wide range of reported concentrations that would not necessarily be clear in a narrative review. We show that meta-regression, including experimental methods as moderators, can be used as an alternative to new animal experiments to answer research questions in the field of neurochemistry. To reach the full analytical potential of this and other types of meta-analyses, the levels of reporting of experimental detail need to improve.

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### Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Appendix S1.** Study characteristics of papers reporting baseline adenosine concentrations.

**Appendix S2.** Study characteristics of papers and abstracts not reporting absolute baseline adenosine concentrations or only reporting them in low-resolution figures.

**Appendix S3.** Adenosine monophosphate concentrations and corresponding study characteristics.

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