## Systematic Review Protocol for Animal Intervention Studies

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<table>
<thead>
<tr>
<th>Item #</th>
<th>Section/Subsection/Item</th>
<th>Description</th>
<th>Check for approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. General</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Title of the review</td>
<td>Amino acids in microdialysates</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Authors (names, affiliations, contributions)</td>
<td>Cathalijn H.C. Leenaars Judith van Luijk Jennifer Freymann Thomas J. van Ee Bea Zoer Pim Drinkenburg Rob B.M. de Vries</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Other contributors (names, affiliations, contributions)</td>
<td>Sophie Jansen and A. Offens are kindly acknowledged for providing assistance in protocol development.</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Contact person + e-mail address</td>
<td><a href="mailto:Cathalijn.Leenaars@radboudumc.nl">Cathalijn.Leenaars@radboudumc.nl</a></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Funding sources/sponsors</td>
<td>ZonMW</td>
<td></td>
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<tr>
<td>6.</td>
<td>Conflicts of interest</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Date and location of protocol registration</td>
<td>12-JAN-2017 SYRCLE website</td>
<td></td>
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<tr>
<td>8.</td>
<td>Registration number (if applicable)</td>
<td>-</td>
<td></td>
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<tr>
<td>9.</td>
<td>Stage of review at time of registration</td>
<td>Searches in progress</td>
<td></td>
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<tr>
<td>B. Objectives</td>
<td></td>
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<td>10.</td>
<td>What is already known about this disease/model/intervention? Why is it important to do this review?</td>
<td>Since its development in the 1980’s, microdialysis has widely been used for <em>in vivo</em> measurement of neurotransmitters and neuromodulators at the locations where they are present.1 Amino acids are not only the building blocks of proteins, but can also function as excitatory (e.g. Glu and Asp) and inhibitory (GABA) neurotransmitters, and have habitually been measured with microdialysis in several regions of the brain. Preceding narrative reviews have e.g. analysed the neuronal vs. glial origin of Glu and GABA in dialysates2, or described the effects of anaesthesia on several amino acids in dialysates3. Microdialysis concentration measurements heavily depend on experimental factors such as the perfusion fluid running speed, the used membrane and the region of interest. Methods vary between research groups, which may result in differences in baseline values. To date, no systematic review of the microdialysis technique has been published. This systematic review will map all published baseline amino acid concentrations in dialysate for the selected amino acids, and explore variability within and between research groups.</td>
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<td></td>
<td>Research question</td>
<td></td>
<td></td>
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<tr>
<td>11.</td>
<td>Specify the disease/health problem of interest</td>
<td>Any (no restrictions)</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Specify the population/species studied</td>
<td>All non-human animals</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Specify the intervention/exposure</td>
<td>Microdialysis as a measurement method Otherwise: Any or none (baseline)</td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>Specify the control population</td>
<td>Any</td>
<td></td>
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<tr>
<td>15.</td>
<td>Specify the outcome measures</td>
<td>Concentration of the amino acids asparagine, aspartate, GABA, glutamate, glutamine, glycine, histamine, proline and taurine in dialysate</td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>State your research question (based on items 11-15)</td>
<td>What is the reported range of concentrations in intracerebral microdialysates for the amino acids asparagine, aspartate, GABA, glutamate, glutamine, glycine, histamine, proline and taurine?</td>
<td></td>
</tr>
</tbody>
</table>

### C. Methods

#### Search and study identification

| 17. | Identify literature databases to search (e.g. Pubmed, Embase, Web of science) | X MEDLINE via PubMed □ Web of Science □ SCOPUS X EMBASE □ Other, namely: □ Specific journal(s), namely: |
| 18. | Define electronic search strategies (e.g. use the step by step search guide\textsuperscript{15} and animal search filters\textsuperscript{20, 21}) | Search strategy provided below protocol table |
| 19. | Identify other sources for study identification | □ Reference lists of included studies □ Books □ Reference lists of relevant reviews □ Conference proceedings, namely: □ Contacting authors/ organisations, namely: □ Other, namely: X None (Our preceding review of adenosine in intracerebral microdialysates shows negligible added value) |
| 20. | Define search strategy for these other sources | - |

#### Study selection

| 21. | Define screening phases (e.g. pre-screening based on title/abstract, full text screening, both) | 1. title/abstract screening 2. full text screening |
| 22. | Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved | a) Two independent reviewers per screening phase (both phases will use EROS) b) Discussion until concession is reached |

**Define all inclusion and exclusion criteria based on:**

| 23. | Type of study (design) | Inclusion criteria: Primary study Exclusion criteria: Review not including new data |
| 24. | Type of animals/population (e.g. age, gender, disease model) | Inclusion criteria: Any animal Exclusion criteria: Human study, in vitro study |
| 25. | Type of intervention (e.g. dosage, timing, frequency) | Inclusion criteria: Intracerebral microdialysis Exclusion criteria: Extracerebral dialysis, no microdialysis |
| 26. | Outcome measures | Inclusion criteria: baseline asparagine AND/OR aspartate AND/OR GABA AND/OR glutamate AND/OR glutamine AND/OR glycine AND/OR histamine AND/OR proline AND/OR taurine in dialysate Exclusion criteria: None of the selected amino acids measured in
## Language restrictions

**Inclusion criteria:** Any  
**Exclusion criteria:** -

## Publication date restrictions

**Inclusion criteria:** Any  
**Exclusion criteria:** -

## Other

**Inclusion criteria:** -  
**Exclusion criteria:** -

## Sort and prioritize your exclusion criteria per selection phase

### Selection phase: Screening title/abstract
1. No microdialysis  
2. Extracerebral dialysis  
3. Human & in vitro studies  
4. None of the selected amino acids measured in dialysate (asparagine AND/OR aspartate AND/OR GABA AND/OR glutamate AND/OR glutamine AND/OR glycine AND/OR histamine AND/OR proline AND/OR taurine)

### Selection phase: Full text
1. No primary study or review without new data  
2. No microdialysis  
3. Extracerebral  
4. Human & in vitro studies  
5. None of the selected amino acids measured

## Study characteristics to be extracted (for assessment of external validity, reporting quality)

### Study ID (e.g. authors, year)
- Authors  
- Year  
- Title  
- Journal  
- Language  
- Research group  
- Laboratory location

### Study design characteristics (e.g. experimental groups, number of animals)
- Number of animals

### Animal model characteristics (e.g. species, gender, disease induction)
- Animal species/strains  
- Age/weight  
- Sex

### Intervention characteristics (e.g. intervention, timing, duration)
- Flow rate  
- Probe length  
- Probe / membrane type  
- Probe location (brain area)  
- Re-use of probe or animal  
- Washout time  
- Baseline measurement time  
- Type of anaesthesia/freely behaving

### Outcome measures
Baseline concentration of the amino acids asparagine, aspartate, GABA, glutamate, glutamine, glycine, histamine, proline and taurine in dialysates (nmol/ml; refer to 39)

### Other (e.g. drop-outs)
- 

## Assessment risk of bias (internal validity) or study quality

### Specify (a) the number of reviewers assessing the risk of bias/study quality
(a) 1 reviewer; a random sample of approximately 5% of the included studies will be checked by a second
in each study and (b) how discrepancies will be resolved

(b) Discussion between reviewers

38. Define criteria to assess (a) the
internal validity of included studies
(e.g. selection, performance, detection and attrition bias) and/or
(b) other study quality measures (e.g. reporting quality, power)

☐ By use of **SYRCLE’s Risk of Bias tool**

☐ By use of SYRCLE’s Risk of Bias tool, adapted as follows:

☐ By use of **CAMARADES’ study quality checklist, e.g.**

☐ By use of CAMARADES’ study quality checklist, adapted as follows:

☐ Other criteria, namely: Extracted study characteristics (31-35)
will be tabulated. This information (or lack of it) provides an indication of study quality, internal validity and risk of bias. (The available risk of bias tools are hardly applicable to baseline measurements.)

**Collection of outcome data**

39. For each outcome measure, define
the type of data to be extracted (e.g. continuous/dichotomous, unit of measurement)

Baseline concentration of the amino acids asparagine, aspartate, GABA, glutamate, glutamine, glycine, histamine, proline and taurine in dialysates in nmol/ml. Where needed, concentration units will be converted.

40. Methods for data extraction/retrieval
(e.g. first extraction from graphs using
a digital screen ruler, then contacting authors)

☐ Data extraction from tables and text

☐ If only graphical data is available, digital image software will be used to obtain these data

41. Specify (a) the number of reviewers
extracting data and (b) how
discrepancies will be resolved

a) 1 reviewer; a random sample of approximately 5% of
the included studies will be checked by a second
reviewer.

b) Discussion between reviewers

**Data analysis/synthesis**

42. Specify (per outcome measure) how
you are planning to combine/compare
the data (e.g. descriptive summary,
meta-analysis)

Results will be tabulated, grouped by laboratory. No meta-
analysis is planned.

| Final approval by (names, affiliations): | Cathalijn Leenaars (SYRCLE) | Rob de Vries (SYRCLE) | Date:12-JAN-2017 |

**References:**


Search strategy:

**PubMed**

**Amino Acids**

**Asparagine**

**Aspartate**

**GABA**

**Glutamate**

**Glutamine**

**Glycine**

**Histamine**

**Proline**

**Taurine**

**Microdialysis**


**Embase**

**Amino Acids**
amino Acid/ OR ((Amino OR Aminergic)AND (Acid OR Acids)).ti,ab,kw. OR

**Asparagine**
asparagine/ OR (Asparagine* OR Asn OR (carbamoylpropanoic AND (Acid OR Acids))).ti,ab,kw. OR

**Aspartate**
aspartic Acid/ OR (Aspart* OR ((Aspar* OR Aminosuccinic) AND (Acid OR Acids)) OR Asp).ti,ab,kw. OR
GABA
4 aminobutyric Acid/ OR GABA.ti,ab,kw. OR ((Aminobut* OR Butanoic OR Butyric) AND (Acid OR Acids)).ti,ab,kw. OR
Glutamate
 glutamic Acid/ OR Glutamate.ti,ab,kw. OR Glu.ti,ab,kw. OR ((Glutamic OR Glutaric OR Aminopentanedioic) AND (Acid OR Acids)).ti,ab,kw. OR
Glutamine
 glutamine/ OR (glutamine OR Gln OR (carbamoylbutanoic AND (Acid OR Acids))).ti,ab,kw. OR
Glycine
glycine/ OR (Glycine* OR Gly OR (Aminoacetic AND Acid) OR glycocoll).ti,ab,kw. OR
Histamine
 histamine/ OR (Histamine OR His OR ethanamine OR Histidine).ti,ab,kw. OR
Proline
 proline/ OR (Proline OR Pro).ti,ab,kw. OR
Taurine
taurine/ OR (Taurine OR Tau OR ((Tauric OR aminoethanesulfonic OR aminoethane) AND (Acid OR Acids))).ti,ab,kw. AND
Microdialysis
 microdialysis/ OR (micro dial* OR microdial* OR microD OR chemitrode OR dialytrode OR brain dialys* OR intracerebral dialys* OR cerebral dialys* OR intracranial dialys* OR cranial dialys* OR transcranial dialys*).ti,ab,kw.

AND the **SYRCLE animal filter** (de Vries, R., et al., Updated version of the Embase search filter for animal studies. 2014.)