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**Characterising the role of the neuropeptide  
substance P in experimental subarachnoid  
haemorrhage**

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for the degree of Doctor of Philosophy

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## DECLARATION

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Christine Barry

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## PUBLICATIONS

The following articles have been published or accepted for publication during the period of my PhD candidature, and sections of these articles are included in the present thesis.

### *Published papers*

**Barry C, Helps S, van den Heuvel C and Vink R (2011)** Characterizing the role of the neuropeptide substance P in experimental subarachnoid haemorrhage. *Brain Research*  
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## ***ABBREVIATIONS***

°C	degrees Celsius
µL	micro litres
µm	micrometres
5-HT	5-Hydroxytryptamine (serotonin)
ACE	angiotensin converting enzyme
aCSF	artificial cerebrospinal fluid
ANOVA	analysis of variance
ATP	adenosine triphosphate
BBB	blood-brain barrier
BP	blood pressure
CBF	cerebral blood flow
CGRP	calcitonin gene-related peptide
cm	centimetres
CNS	central nervous system
CPP	cerebral perfusion pressure
CSD	cortical spreading depolarisation
CSF	cerebrospinal fluid
d	day
DAB	diaminobenzidine
eNOS	endothelial nitric oxide synthase
G	gauge
h	hours

## Abbreviations

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H and E	haematoxylin and eosin
H <sup>+</sup>	hydrogen ion
ICA	internal carotid artery
ICH	intracerebral haemorrhage
ICP	intracranial pressure
icv	intracerebroventricular
iv	intravenous
IU	international units
K <sup>+</sup>	Potassium ion
L/min	litres per minute
MABP	mean arterial blood pressure
MCA	middle cerebral artery
mg/ml	milligrams per millilitre
mins	minutes
ml	millilitres
mm	millimetres
mmHg	millimetres of mercury
mRNA	messenger ribonucleic acid
n	number
NAT	n-acetyl-L-tryptophan
NEP	neutral endopeptidase
NK1	tachykinin receptor to which SP binds selectively
NK2	tachykinin receptor to which NKA binds selectively
NK3	tachykinin receptor to which NKB binds selectively

## Abbreviations

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NKA	neurokinin A
NKB	neurokinin B
NMDA	N-methyl-D-aspartate
NO	nitric oxide
pCO <sub>2</sub>	carbon dioxide partial pressure
PNS	peripheral nervous system
pO <sub>2</sub>	oxygen partial pressure
PPT	preprotachykinin
rpm	revolutions per minute
Rx	treatment
s	seconds
SAH	subarachnoid haemorrhage
SEM	standard error of the mean
SD	standard deviation
SP	substance P
TRPV1	transient receptor potential vanilloid 1



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## ABSTRACT

### Background

Raised intracranial pressure (ICP) following SAH predicts poor outcome and is due to hemorrhage volume and possibly brain oedema, hydrocephalus and increased volume of circulating intracranial blood. Interventions that reduce oedema may therefore reduce ICP and improve outcome. The neuropeptide substance P (SP) mediates vasogenic oedema formation in animal models of ischemic stroke, intracerebral hemorrhage and brain trauma, and may contribute to the development of increased ICP. Blockade of the SP NK1 tachykinin receptor using n-acetyl-l-tryptophan (NAT) reduces brain oedema and improves outcome in these models. This intervention had not previously been tested in models of SAH. This study therefore assessed whether SP mediates oedema formation in experimental SAH, and whether NAT treatment impacted on ICP and functional outcome.

### Methods

SAH was induced in adult male Sprague-Dawley rats by either injection of autologous blood into the prechiasmatic cistern (injection SAH) or by endovascular arterial puncture of the Circle of Willis (filament SAH). NAT was injected (i.v.) at 30 minutes after induction of SAH. Subgroups were assessed for brain water content, immunoreactivity to SP, albumin immunoreactivity and functional outcome at 5, 24 and 48 hours, or ICP and cerebral perfusion pressure during SAH and over the following 5 hours.

## **Results**

In both models a primary ICP increase occurred during SAH and a secondary ICP increase occurred within 2 hours. Injection SAH was followed by a non-significant increase in brain water content and caused no functional deficits. In contrast, brain oedema followed filament SAH ( $p < 0.001$ ) and correlated with functional deficits ( $r = 0.8, p < 0.01$ ). Increased albumin immunoreactivity ( $p < 0.001$ ) indicated vasogenic brain oedema. Cerebral perfusion pressure was diminished after filament SAH and some animals demonstrated plateau waves of ICP. NAT treatment did not improve ICP, oedema or outcome.

## **Conclusion**

SAH produced secondary ICP elevation, vasogenic brain oedema and functional deficits, but it is unclear if oedema contributed to ICP. Blockade of SP did not improve any outcome parameters, suggesting that SP-mediated neurogenic inflammation may be less critical to outcome than other factors in these models.