UNIVERSIDADE DO SUL DE SANTA CATARINA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE
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O PAPEL DA ATIVAÇÃO DO SISTEMA IMUNE NEONATAL NO DESENVOLVIMENTO DE UM COMPORTAMENTO RELACIONADO A ESQUIZOFRENIA: UM ESTUDO PRÉ-CLÍNICO

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LINHA DE PESQUISA: NEUROCIÊNCIAS

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Orientadora: Profa. Dra. Clarissa Martinelli Comim

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O papel da ativação do sistema imune neonatal no desenvolvimento de um comportamento relacionado a esquizofrenia: um estudo pré-clínico

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“Se vi mais longe, foi porque estava sobre os ombros de gigantes”

Issac Newton

RESUMO

Introdução: Apesar dos avanços nos cuidados médicos, a ativação imune neonatal continua a ser uma causa comum e significativa de mortalidade e morbidade entre crianças. A ativação do sistema imunológico durante o início da vida foi associada a um risco aumentado de esquizofrenia na idade adulta.

Objetivo: O objetivo deste estudo foi avaliar o comportamento semelhante à esquizofrenia em camundongos adultos após a ativação imune neonatal.

Métodos: Camundongos C57BL/6 machos e fêmeas neonatais nos dias pós-natal 2-3 receberam uma injeção de 25 µg de lipopolissacarídeo (LPS) ou PBS como um placebo. O comportamento semelhante à esquizofrenia foi induzido por cetamina (25, 50 ou 100 mg/kg) no dia 28 pós-natal. Testes de atividade locomotora, comportamento estereotipado e interações sociais foram conduzidos 30 min após a injeção de cetamina ou solução salina.

Resultados: Camundongos adultos jovens que receberam cetamina na dose de 50 mg/kg apresentaram aumento da atividade locomotora; escores de estereótipo e latência de contato também foram significativamente maiores em comparação com o grupo de controle que recebeu a mesma dose de cetamina.

Conclusão: Concluímos que a exposição à ativação neonatal imune durante o período neonatal pode causar alterações no desenvolvimento normal do cérebro e desencadear um comportamento semelhante à esquizofrenia na idade adulta.

Descritores: Ativação imune neonatal. Comportamento relacionado a esquizofrenia. Transtornos psiquiátricos. LPS.

ABSTRACT

Introduction: Despite advances in medical care, neonatal immune activation continues to be a common and significant cause of mortality and morbidity among infants. Activation of the immune system during early life has been associated with an increased risk of schizophrenia in adulthood.

Objective: The aim of this study was to evaluate schizophrenia-like behaviour in adult mice following neonatal imune activation.

Methods: Neonatal male and female C57BL/6 mice at postnatal days 2-3 received an injection of 25 µg of lipopolysaccharide (LPS) or PBS as a placebo. Schizophrenia-like behaviour was induced by ketamine (25, 50, or 100 mg/kg) at postnatal day 28. Tests of locomotor activity, stereotyped behaviour, and social interactions were conducted 30 min after injection of ketamine or saline.

Results: Young adult mice that received ketamine in a dose of 50 mg/kg showed an increase in locomotor activity; stereotype scores and contact latency were also significantly higher compared with the control group that received the same ketamine dose.

Conclusion: We conclude that exposure to imune neonatal activation during the neonatal period can cause alterations in normal brain development and trigger schizophrenia-like behaviour in adulthood.

Keywords: Imune neonatal actiation. Schizophrenia-like behaviour. Psychiatric disorders. LPS.

LISTAS

Lista de abreviaturas

ABP- Associação Brasileira de Psiquiatria

ANOVA – Análise de Variância

AVAIs - Anos de vida ajustados para a incapacidade

AVI - Anos de vida com incapacidade

ATP - Adenosina trifosfato

CEUA - Comissão de Ética no Uso de Animais

CFMV - Conselho Federal de Medicina Veterinária

CONCEA - Conselho Nacional de Controle de Experimentação Animal

EUA – Estados Unidos da América

FAPESC - Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina

IL- Interleucina

LANEX – Laboratório de Neurociências Experimental

LPS – Lipopolissacarídeos

MIA – Morte Indolor Assistida

NIH - Instituto Nacional de Saúde dos EUA

NMDAR – receptor N-metil-D-aspartato

OMS - Organização Mundial de Saúde

PBS - Tampão fosfato

SNC - Sistema nervoso Central

TEA - Transtorno do Espectro Autista

TNF – Fator de Necrose Tumoral

UFSC - Universidade Federal de Santa Catarina

UNISUL – Universidade do Sul de Santa Catarina

Lista de quadros

SUMÁRIO

1. INTRODUÇÃO

As psicopatias são transtornos que há muito tempo vem desafiando o mundo científico, podendo gerar prejuízos permanentes tanto na vida do indivíduo diagnosticado quanto na vida dos indivíduos que se encontram próximo a ele[1]. Conforme a definição do dicionário, esse distúrbio mental é descrito como grave em que o portador possui uma conduta antissocial e amoral, sem demonstração de arrependimento, inapto para amar e se relacionar com outros seres humanos com laços afetivos intensos, individualistas ao extremo e inaptidão de aprendizado com a experiência[2]. Devido a evolução científica e embasamento também nos aspectos sociais e morais, as psicoses podem ser definidas como aquelas que apresentam como característica comum a desorganização de pensamentos com perda da associação de ideias, o que leva a dificuldade de execução de tarefas rotineiras e distanciamento das relações interpessoais[3]. Dentre os transtornos mentais graves, a esquizofrenia é uma das mais relevantes por acometer aproximadamente 1% da população mundial[4] e por ser altamente incapacitante[5–7]. Esses transtornos são responsáveis por 1,1% dos AVAIs (anos de vida ajustados para incapacidade) e por 2,8% dos AVIs (anos de vida com incapacidade), o que os torna de extrema relevância para a saúde pública[8]. Geralmente costuma aparecer como surto psicótico[9], que inicia na adolescência tardia ou início da fase adulta[,6,10], ocorrendo no sexo masculino por volta dos 20 anos e no feminino aos 25 anos, podendo se perpetuar ao longo da vida[9].

A esquizofrenia é classificada pela psiquiatria como sendo a única doença que apresenta a condição de psicose. Atualmente não há cura, embora possa haver remissão dos sinais ou ser controlada com medicação[11], possui a perda do aspecto essencial do ser humano (fundamento social)[12]. É um Transtorno mental crônico com evolução heterogênea, caracterizado por diversos sintomas os quais são subdivididos em positivos e negativos, respectivamente, por aumento e redução de dopamina nas vias dopaminérgicas [8,13–15]. Os sintomas denominados de positivos incluem alucinações auditivas, delírios, desordem mental, crença paranoide e períodos de remissão [6,16]. Os sintomas negativos incluem estereotipias, déficit de memória, alterações da sensopercepção, emoções distorcidas da realidade ou dificuldade no reconhecimento das emoções, avolia, discursos abreviados por

comprometimento da linguagem[7,9,16]. Além disso, inclui os distúrbios cognitivos [17,18], como déficits na capacidade de aprender, presença de ansiedade, culpabilidade, depressão e autopunição acarretando tentativas de suicídio[7,15].

A intensidade dos sintomas, tanto positivos quanto negativos, pode variar conforme o curso da doença, prejudicando assim todas as relações familiares e sociais do indivíduo, causando um grande sofrimento psíquico [9,19]. Além disso, exames patológicos evidenciaram que o encéfalo de indivíduos com diagnóstico de esquizofrenia é mais leve e de menor tamanho quando comparado ao de pessoas incluídas em grupos de controles. Também foram encontradas alterações nos lobos temporais, principalmente no hipocampo e giro para-hipocampal. Estas estruturas estão relacionadas principalmente a cognição e aos processos de memória e aprendizagem[9,19].

Neste contexto, estudos têm mostrado uma relação importante entre transtornos psiquiátricos associados a declínios cognitivos e processos inflamatórios sistêmicos. Nesta mesma perspectiva, pesquisas realizadas em sujeitos diagnosticados com a patologia revelaram uma desordem na resposta imunológica relacionada ao aumento nos níveis de citocinas pró-inflamatórias, o que sugere mais uma vez o envolvimento do processo de neuroinflamação[20–22].

Levando-se em conta o exposto acima, sabe-se que a esquizofrenia é um transtorno psiquiátrico que afeta de forma grave a qualidade de vida tanto do indivíduo afetado quanto de seus familiares e círculo social, além de impactar de forma considerável nos gastos públicos com saúde. Sabendo-se que as intervenções existentes na atualidade visam tratar e controlar as diferentes comorbidades envolvidas nesse transtorno, mas não são efetivamente uma cura para a doença. Considerando que o desenvolvimento de modelos animais consistentes e validados possibilitam a melhor compreensão das diferentes facetas tanto do desenvolvimento quanto da progressão da esquizofrenia. O uso combinado de dois modelos animais passíveis de desencadear as características da esquizofrenia são de extrema importância para melhor compreensão da etiologia da doença.

Neste sentido, a aplicação de um modelo o qual mimetiza a instalação de um processo inflamatório durante o neurodesenvolvimento inicial, combinado com administração de cetamina ao longo da vida, permite a projeção de projetos posteriores com o intuito de investigar os processos que levam ao desenvolvimento

da esquizofrenia durante a fase prodrômica da doença, retardar o aparecimento dos sintomas, além de potencializar o desenvolvimento de intervenções que possam aliviar as alterações neuroquímicas e comportamentais, visando impedir a progressão para a psicose e melhorar a qualidade de vida dos indivíduos afetados e seus familiares.

Destaca-se ainda que essa pesquisa possui como uma de suas caraterísticas o fator extraordinário devido realizar a associação de dois modelos, sendo que um modelo (alteração no período de desenvolvimento) predispõe a antecipação de um segundo modelo (induzido por drogas - cetamina). Além de utilizar doses menores de cetamina descritas na literatura afim de resultar no desfecho do transtorno.

Por fim, este estudo busca elucidar a questão: qual o possível papel da ativação do sistema imune neonatal no modelo animal de esquizofrenia induzida por administração de cetamina? Como Hipótese principal, considera-se que a ativação imune neonatal leva a um processo de neuroinflamação no sistema nervoso em desenvolvimento, podendo resultar como o desfecho, o próprio transtorno após a exposição a um segundo fator de risco (cetamina) ao longo da vida.

1.1 REFERENCIAL TEÓRICO

1.1.1 Esquizofrenia

A Esquizofrenia é considerada um transtorno complexo com déficits de conectividade neural devido a alterações em regiões-chave de circuitos neuronais. Estudos mostram que alterações no córtex pré-frontal e no cerebelo foram ligadas a sintomas negativos e déficits no circuito córtico-cerebelar-talâmico-cortical foram associadas a alterações cognitivas[23,24]. Atualmente é caracterizada como um transtorno crônico e debilitante que afeta pelo menos 1% da população em todo o mundo, sendo classificado pelo Organização Mundial de Saúde (OMS) como uma das 10 maiores causas de incapacidade[6]. Os sintomas da esquizofrenia, os quais se manifestam tipicamente durante a adolescência e início da idade adulta, geralmente se enquadram em um dos três grupos: positivos (por exemplo, alucinações e delírios), negativos (por exemplo, isolamento social, anedonia) e cognitivos (por exemplo, disfunção de processamento sensorial, deficiências na memória de trabalho)[6,16,18].

A origem etiopatológica da esquizofrenia é ainda considerada idiopática, embora estudos apontem correlação multifatorial, esse transtorno pode ser causado por elementos biopsicossociais, também conhecidos como fatores de vulnerabilidade, que interagem designando circunstâncias favoráveis ou não a manifestação do distúrbio[25,26].

Os fatores biológicos estão correlacionados a genética e também podem estar ligados a danos ou anomalias no encéfalo, bem como deficiências nos níveis e na função de diferentes neurotransmissores[27,28]. Além disso, a literatura, desde a década de 80, também aponta a hipótese de neurodesenvolvimento como fator de risco para o surgimento deste transtorno, partindo do princípio em que ocorram insultos cerebrais primários durante o início da formação cerebral, muito antes da patologia se evidenciar clinicamente[21]. Progressos atuais nas técnicas de imagem cerebral fortaleceram a hipótese de neurodesenvolvimento da esquizofrenia, salientando a relevância das alterações cerebrais gradativas que ocorrem ao longo das fases iniciais da doença, ou seja, antes e/ou durante a transição para psicose desenvolvida [29,30]. Sendo assim, as alterações cerebrais nesse transtorno aparentam ser mais dinâmicas do que se suspeitava antecedentemente, de maneira que uma interação entre distúrbios do neurodesenvolvimento precoce e episódios patológicos que acontecem ao longo da maturação cerebral posterior ao nascimento parece essencial para estimular a manifestação da esquizofrênica evidente[31–34]. Ainda nesse contexto, existem as evidências de alterações de fatores imunológicos envolvidos na etiologia e fisiopatologia da esquizofrenia[35,36], como o mecanismo da neuroinflamação do desenvolvimento, que pode predispor o organismo a mudanças cerebrais e comportamentais consideráveis para a esquizofrenia[37–39].

Os fatores psicossociais, os quais consistem no ponto de vista psicológico do indivíduo e interação com o ambiente social, também estão relacionados com a esquizofrenia, ou seja, pessoas predispostas a patologia podem desenvolver a enfermidade quando estimuladas por fatores tanto internos quantos externos[40,41]. Neste sentido, sabe-se que o risco de cada indivíduo para o desenvolvimento de esquizofrenia depende do tipo de ambiente ao qual está exposto e, pelo menos em parte, do seu próprio nível individual de desvantagem social, como desemprego, nível de educação reduzido, condições precárias de habitação, dentre outros [42].

Neste contexto, a esquizofrenia e a depressão maior se enquadram entre os dez principais transtornos mentais que geram incapacidade e reduzem o tempo de

vida. Tomados em conjunto, no ano 2000 esses dois transtornos contribuíram em torno de 11,2% para a carga global de doenças na população com faixa etária entre 15 e 44 anos[43].

O Conselho Nacional de Saúde aponta que 23 milhões de brasileiros possuam algum transtorno mental, dos quais 5 milhões sofrem de perturbações constantes e severas[44]. De acordo com a Associação Brasileira de Psiquiatria (ABP), a política de saúde mental prioriza a esquizofrenia e o transtorno bipolar como as doenças mais relevantes[44]. Pondera-se que os transtornos esquizofrênicos acometam de 0,6 % a 3% da população mundial, estimando que a incidência deve estar entre 1 e 7 novos casos anuais para cada 10.000 habitantes[45–47], embora muitos casos não sejam documentados por falta de diagnóstico clínico. Além disso, pacientes com esquizofrenia sofrem do aumento de morbidade e mortalidade em relação à população geral, tendo uma expectativa de vida reduzida em 20% [48].

A esquizofrenia de início muito precoce, ou seja, com idade inferior a 13 anos é considerada incomum, embora existam alguns relatos na literatura com idade inferior a 5 anos. Estima-se que cerca de 0,1 a 1% dos casos desse transtorno tenha começado antes dos 10 anos de idade e cerca de 4% antes dos 15 anos[49,50]. Embora a saúde pública tenha se aprimorado nos últimos anos ainda são poucos os estudos epidemiológicos realizados no Brasil, o que não exclui a importância da doença e sim realça a dificuldade de realizar esses tipos de estudos, ressaltando assim a importância de realizar novas pesquisas. Nesse sentido, especula-se que as estimativas de incidência e prevalência sejam compatíveis com as observadas em outros países[47]. Pesquisas realizadas em três capitais brasileiras, no ano 1992, obtiveram como resultados a prevalência de 0,3 % a 2,4 % para psicoses[51]. Outro estudo, realizado no ano 2000, no Rio Grande do Sul, mostra as psicoses como um dos principais diagnósticos em internações hospitalares[52].

Além disso, após as alterações nos critérios de diagnóstico psiquiátrico, no ano de 2004, a prevalência foi de aproximadamente 20% das internações no estado do Rio Grande do Sul[52]. Uma pesquisa publicada no ano de 2007, conduzida na cidade de São Paulo, a maior metrópole brasileira, identificou 367 casos classificados como primeiro episódio psicótico, dentre os quais 51% eram mulheres e quase 40% preencheram os critérios de esquizofrenia[53]. Neste estudo, a taxa de incidência anual de qualquer psicose foi de 15,8 para cada 100.000 pessoas dentre o grupo de risco (18 a 64 anos). Além disso, a incidência de psicoses não afetivas foi

maior entre os homens mais jovens e, curiosamente, observaram menores taxas de incidência de esquizofrenia nesse ambiente urbano em comparação com outras grandes cidades[53], o que levanta a discussão de que os processos socioambientais envolvidos na etiologia da esquizofrenia possam ser mais complexos do que simples associações lineares com a urbanidade[54].

Em relação à prevalência entre os sexos a maior parte dos estudos não relata divergência[47], se difere apenas no início dos sinais clínicos da doença sendo mais precoce no sexo masculino [25]. Apesar de a incidência deste transtorno se apresentar de forma semelhante entre homens e mulheres, estudos mostram que dentre os portadores de esquizofrenia internados em clínicas psiquiátricas existe uma porcentagem maior de homens [46,55,56] sugerindo que os desfechos a longo prazo podem ser mais graves no sexo masculino, considerando, dentre outros critérios, índices de mortalidade e suicídio[46].

No contexto patognomônico, a principal característica dessa doença neuropsiquiátrica consiste na dificuldade de discernir o pensamento da realidade. Geralmente os indivíduos acometidos não possuem perda de consciência e nem desprovimento de intelecto. Os sintomas podem ser divididos em dois conjuntos: positivos e negativos. Os positivos consistem nas distorções do pensamento ou na exacerbação dos estímulos externos bem como delírio, alucinação, discurso desorganizado e catatonia. Já os sintomas negativos são caracterizados por embotamento do afeto, alogia e avolia[57].

Atualmente o diagnóstico é elaborado através da 5ª edição do Manual diagnóstico e Estatístico de Transtornos Mentais (DSM-V), o qual estipula os mesmos critérios de diagnóstico para crianças, adolescentes e adultos. De acordo com esse documento, para efetivo diagnóstico de esquizofrenia, o indivíduo precisa ter transtornos psicóticos[57], em que exista uma deformidade do pensamento por um período de seis meses e um período de um mês na fase ativa com dois ou mais sintomas. O diagnóstico é realizado somente por psiquiatras de forma clínica e com fundamentação na anamnese do paciente. O diagnóstico precoce aumenta as chances de uma melhor qualidade de vida e convívio social. Todavia, existem muitos empecilhos para concluir essa análise, especialmente na infância, podendo ocorrer sobreposição de sintomas com outras enfermidades psiquiátricas, acarretando, em muitos diagnósticos imprecisos pela questão de a criança ser um indivíduo em desenvolvimento[49,50].

1.1.2 Esquizofrenia e Neuroinflamação

A correlação entre inflamação e psicopatologias foi primordialmente expressa através de pesquisas epidemiológicas em que epidemias de gripe em gestantes encabeçaram aumentos consideráveis nos transtornos do espectro da esquizofrenia nos seus descendentes[58]. Dessa maneira, a inflamação durante a gestação passou a ter maior relevância no desenvolvimento de enfermidades neurológicas, abrangendo os transtornos de humor e transtornos do espectro do autista (TEA)[21,59,60]. Diversos estudos demonstraram que infecções por microrganismos, sejam elas, bacterianas ou virais, durante o período gestacional, estão relacionadas a processos de neuroinflamação na fase intrauterina do indivíduo devido ativação do sistema imune, aumentando assim as chances de desenvolver esquizofrenia[23,59,60].

Numerosos estudos já discutiram a participação das doenças infecciosas tanto em neonatos quanto em adultos, para o risco de desenvolvimento de transtornos psiquiátricos[61,62]. Uma pesquisa revelou que crianças internadas por uma infecção bacteriana no SNC entre as idades de 2 e 5 anos encontram um risco quatro vezes maior de hospitalização tardia para transtornos psiquiátricos como a depressão e a esquizofrenia[63]. Outro estudo, sobre as doenças infecciosas e transtornos psiquiátricos, relatou que os casos de meningite aguda, encefalite, febre tifóide, febre recorrente, tifo, gripe, malária, tuberculose, infecções gastrointestinais e septicemia foram frequentemente seguidos por um episódio psiquiátrico agudo transitório[60]. Uma pesquisa longitudinal, comparando indivíduos com infecção no SNC durante a infância com um grupo controle acompanhados até 27 anos de idade, encontrou um aumento nos casos de esquizofrenia no grupo infecção[64]. Além disso, sintomas de esquizofrenia já foram observados em diferentes doenças inflamatórias, como infecções virais, encefalite recorrente do sarampo[65]; doenças autoimunes e lúpus eritematoso sistêmico[66].

Vale ressaltar que o SNC possui um sistema imune intrínseco estruturado por células de defesa como as microglias e os astrócitos[67–69]. Devido à barreira hematoencefálica, as respostas às infecções e inflamações são divergentes das geradas no sistema nervoso periférico, não apresentando dessa forma os quatro sinais clássicos da inflamação, que são vermelhidão, edema, calor e dor.

Adicionalmente, a resposta dos leucócitos é mais lenta e menos robusta no SNC em comparação à periferia, onde o seu recrutamento costuma ocorrer rapidamente[23].

O processo de inflamação do SNC no período fetal pode se manifestar através do sistema imunológico materno, o qual além de afetar o neurodesenvolvimento da prole também pode determinar a progressão da doença[21]. Nesse contexto, o sistema imune quando acionado ativa as células da glia[10,70], as quais possibilitarão mediadores pró-inflamatórios como as citocinas[21,58] que irão atuar na modulação do sistema nervoso fetal[71]. Essa ativação precoce da microglia, pode desequilibrar a regulação do sistema de morte celular programada e refinamento dos contatos sinápticos durante o desenvolvimento, acarretando redução tanto da apoptose quanto da poda sináptica, resultando em aumento no números de sinapses disfuncionais[41]. Assim como, outra via de ativação do sistema imune pode advir por intermédio das células trofoblásticas do embrião as quais em resposta a microrganismos infecciosos liberam citocinas[72]. As principais citocinas liberadas durante esse processo são IL-1β, Fator de Necrose Tumoral (TNF-α) e IL-6 as quais serão processadas pela enzima caspase-1 as tornando maduras para executar as suas funcionalidades[73]. Nesse interim, a literatura médica frequentemente correlaciona que indivíduos com diagnóstico de epilepsia, esquizofrenia e autismo possuem índices elevados dessas mesmas citocinas pró-inflamatórias [74,75].

Quando presentes no SNC, as citocinas pró-inflamatórias ampliam o sinal[60], o que determina a ativação da microglia que por sua vez secreta mais elementos pró-inflamatórios além de quimiocinas e proteases[76]. Em virtude da descompensação das citocinas há um aumento do estresse oxidativo o que eleva os níveis de óxido nítrico. Posteriormente a esse processo ocorre a ativação do eixo hipotálamo-hipófise-adrenal que libera cortisol como resposta[58]. Todo esse processo pode resultar em anormalidades neuronais e por consequência gerar patologias como a esquizofrenia[23].

As células da glia denominadas microglia são os macrófagos residentes no tecido do SNC, são células de defesa as quais exercem o papel nas respostas imunológicas, vigiando e protegendo de forma ativa o tecido encefálico e medular[74]. A microglia tem demonstrado ser um participante ativo em programas complexos do neurodesenvolvimento, como neurogênese e poda sináptica, durante os quais interagem com neurônios e macroglia para fornecer suporte trófico, responder a

citocinas e sinais metabólicos derivados do ambiente neural local e auxiliar no refinamento de circuitos neuronais funcionais[77]. Durante o processo de lesão neurológica ocorre a liberação de adenosina trifosfato (ATP) de neurônios danificados, astrócitos ou células microgliais, fator que ocasiona mudanças fenotípicas na microglia[78], resultando em um formato ameboide e expressando moléculas de superfície, como as proteínas CD14 e receptores de quimiocinas como IL-1β, IL-6, TNF-α. Este é o princípio da resposta inflamatória no SNC, ou neuroinflamação. Quando ocorre a persistência de estímulos prejudiciais tanto internos quanto externos (ambiente) a micróglia os reconhece e gera um sistema de retroalimentação positivo, aumentando os elementos inflamatórios[77].

Neste sentido, fica claro que algumas características da atividade pró-inflamatória finamente reguladas são necessárias para um desenvolvimento neural saudável. No entanto, a inflamação irrestrita no início da vida pode alterar a programação da própria população microglial, culminando em um limiar mais baixo para a reativação, perpetuando danos inflamatórios no compartimento neuronal mais tarde na vida[77]. Quando a microglia é ativada precocemente no SNC imaturo, durante a gestação, pode gerar um processo de memória imunológica e reagir de forma exacerbada a um novo episódio de inflamação e, por consequência, aumentar a probabilidade de desenvolver a esquizofrenia pós infecção[71].

Desta forma, é provável que um aumento do nível de citocinas, durante e após a infecção materna, resultem em efeitos a longo prazo sobre a função encefálica, os quais podem aumentar o risco para o desenvolvimento de transtornos do neurodesenvolvimento, como a esquizofrenia.

1.1.3 Modelos animais de Esquizofrenia

Alguns dos sintomas relacionados à esquizofrenia podem ser mimetizados em animais de laboratório, principalmente em roedores. Fatemi e colaboradores[79,80] realizaram estudos experimentais de exposição pré-natal ao vírus da gripe humana em camundongos e sua relação com o desenvolvimento de esquizofrenia ao longo da vida[79,80]. Em um desses experimentos[79] foi introduzida uma dose subletal de uma cepa neurotrópica do vírus da gripe humana, por via intranasal em camundongos gestantes. Posteriormente foram avaliados os efeitos na prole, tanto cerebrais quanto comportamentais, comparados com o grupo controle. Nesse modelo se

observou também os aspectos morfológicos cerebrais e neuro-anatômicos, evidenciando que a infecção materna induzida por influenza resulta em várias doenças neuropatológicas[79]. Além disso, outro trabalho constatou alterações comportamentais na idade adulta da prole, o que pode estar relacionado aos sintomas positivos e negativos da esquizofrenia[81]. Os camundongos apresentaram déficits em portas sensório-motoras, espaço diminuído de exploração e menor interação social[81].

Os modelos animais comumente utilizados para estudar a esquizofrenia podem ser classificados em quatro categorias distintas: 1) alterações no período do desenvolvimento; 2) induzido por drogas; 3) induzido por lesão ou; 4) por manipulação genética[82–85]. De interesse para este projeto de pesquisa, destaca-se o modelo relacionado ao período do neurodesenvolvimento por meio da ativação imune materna e o modelo induzido por administração de drogas, mais especificamente a cetamina[86]. Os parâmetros analisados nos animais normalmente avaliam comportamentos tipicamente prejudicados em indivíduos esquizofrênicos – como alterações na linguagem, através da análise de vocalizações ultrassônicas, interações sociais, comportamentos de esteriotipias, alterações na atividade locomotora, bem como fenótipos de ansiedade e comprometimento de aprendizagem e memória[87]. Importante salientar que os modelos animais relacionados aos períodos do desenvolvimento que utilizam roedores são hábeis em mimetizar de forma translacional os distintos estágios da gestação humana, embora o tempo gestacional seja bastante diferente[88,89].

Uma gestação de ratos e camundongos costuma girar em torno de 20 dias, sendo que os 10 primeiros dias gestacionais são equivalentes aos 3 primeiros meses da gravidez humana; do dia 10 ao dia 20 em roedores equivale dos 3 aos 6 meses em humanos; e os 10 primeiros dias do período pré-natal em roedores equivalem aos 3 últimos meses da gestação humana[90]. Considerando que a infecção durante a gravidez tem sido associada à esquizofrenia, o modelo de ativação imune neonatal em roedores tornou-se amplamente utilizado para induzir e estudar os fenótipos comportamentais relacionados a esse transtorno psiquiátrico[77]. Estudos tem associado o aumento nos níveis e na expressão de citocinas pró-inflamatórias no soro materno com um comportamento relacionado à psicose em roedores[70].

Além disso, pesquisas em modelos animais evidenciam importantes alterações na prole após injeção de lipopolissacarídos (LPS), um constituinte da parede bacteriana de bactérias Gram-negativas, em ratas prenhas. Os achados demonstram aumento no número de células em apoptose, nos níveis de citocinas pró-inflamatórias com IL-6, IL-1β e TNF-α, ativação microglial ao longo da vida da prole, além de alterações comportamentais, como por exemplo déficits de memória e aprendizagem [91,92,93]. A administração de antagonistas do receptor de glutamato N-metil-D-aspartato (NMDAR), como por exemplo a cetamina, induz transitoriamente sintomas de esquizofrenia aguda e pode ser vinculada a uma disfunção glutamatérgica em modelos farmacológicos de esquizofrenia[86]. A cetamina se liga a uma variedade de receptores, mas atua principalmente no NMDAR, e pesquisas demonstram que existe uma hipofunção deste receptor na esquizofrenia. Essa hipofunção NMDAR, por sua vez, pode explicar anormalidades conectivas e oscilatórias na esquizofrenia em termos de excitação enfraquecida de interneurônios inibitórios gabaérgicos, fato que sincroniza a desinibição de redes neuronais corticais[88]. Além disso, nesse contexto dos NMDAR, um estudo bastante atual em humanos discute evidências de que autoanticorpos NMDAR podem ter um papel importante como um possível biomarcador prognóstico e fator etiológico em pessoas que já atendem aos critérios de alto risco clínico para desenvolver psicoses[94].

De forma geral, os primeiros sintomas ou surto psicótico costumam surgir em indivíduos com predisposição à esquizofrenia após algum evento ao longo da vida, como por exemplo uso de substâncias recreativas[95], que atua como uma espécie de gatilho ambiental. Neste sentido, já está bem estabelecido que o “desafio” ambiental com administração de cetamina é útil para o estudo dos sintomas positivos, negativos e cognitivos da esquizofrenia. Além disso, é possível avaliar através desse modelo animal, a disfunção dopaminérgica e gabaérgica, a idade de início dos sintomas, a desconectividade funcional e as oscilações corticais anormais observadas na esquizofrenia aguda[88,96].

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2. OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar o papel da ativação do sistema imune neonatal no desenvolvimento de um comportamento relacionado a esquizofrenia: um estudo pré-clínico.

2.2 OBJETIVOS ESPECÍFICOS

- Verificar os efeitos de diferentes doses de cetamina sobre a hiperlocomoção em animais adultos jovens submetidos ao modelo de ativação imune neonatal;
- Avaliar os efeitos de diferentes doses de cetamina na relação social em animais adultos jovens submetidos ao modelo de ativação imune neonatal;
- Verificar os efeitos de diferentes doses de cetamina no desenvolvimento de esteriotipia em animais adultos jovens submetidos ao modelo de ativação imune neonatal.

3. MÉTODOS

3.1 TIPO DE ESTUDO

Esta pesquisa foi do tipo experimental pré-clínica. Foi realizada no Laboratório de Neurociências Experimental (LANEX) e no Laboratório de Bioquímica e Biologia Molecular da Universidade do Sul de Santa Catarina (UNISUL), campus Pedra Branca.

3.2 MATERIAIS E EQUIPAMENTOS

Para a realização deste estudo foram utilizados os materiais e equipamentos apresentados no Quadro 1.

Quadro 1 – Materiais e equipamentos utilizados na pesquisa

Materiais e Equipamentos	**Especificação**	**Marca**	**País**
LPS	026:B6L E.Coli	Sigma Aldrich	Brasil
PBS	pH 7.4	Thermo Fisher Scientific	Brasil
Cloridrato de cetamina	50ml	CU Chemie Uetikon	Alemanha
Thiopentax	Pó para solução injetável-1g	Cristália	Brasil
Aparato teste de campo aberto	40x40x40 cm	Marceneiro local	Brasil
Cloridrato de xilazina	10ml	Ceva	Brasil
Cloridrato de dextrocetamina	50ml	Syntec	Brasil
Câmera	Hero 3+	GoPro	EUA

Fonte: autoria própria

3.3 ANIMAIS

Foram utilizados 20 (vinte) camundongos adultos C57BL/6 sendo 10 machos e 10 fêmeas, pesando entre 18 e 22g, totalizando 10 casais provindos da Universidade Federal de Santa Catarina (UFSC). Os animais foram acasalados (um macho para uma fêmea) e a prole utilizada para este estudo. Os animais foram mantidos em caixas de polipropileno, ciclo de claro e escuro de 12 horas (06:00 às 18:00) e comida e água livres. O ambiente foi mantido a temperatura de 23 + 1ºC. Os animais foram acondicionados no Biotério Experimental do LANEX localizado no campus Pedra Branca, no Bloco I2.

O número de animais por grupo foi calculado em n=8. A fórmula empregada para o cálculo foi a equação n/grupo=2[(Zα/2 +Zβ) X d/∆]2, para comparação de duas médias, considerando-se o poder de teste de 80%, o nível de significância de 5%, o desvio padrão de 12,5% a partir de registros de estudos anteriores e o valor da diferença a ser detectada igual a 18%. Entende-se, portanto, que o número de pelo menos 8 animais foi utilizado em cada grupo experimental para garantir que as conclusões dos experimentos sejam válidas, dentro de um risco aceitável de não estar observando diferenças onde elas existam tampouco estar observando diferenças onde elas não existam.

3.4 DELINEAMENTO DO ESTUDO

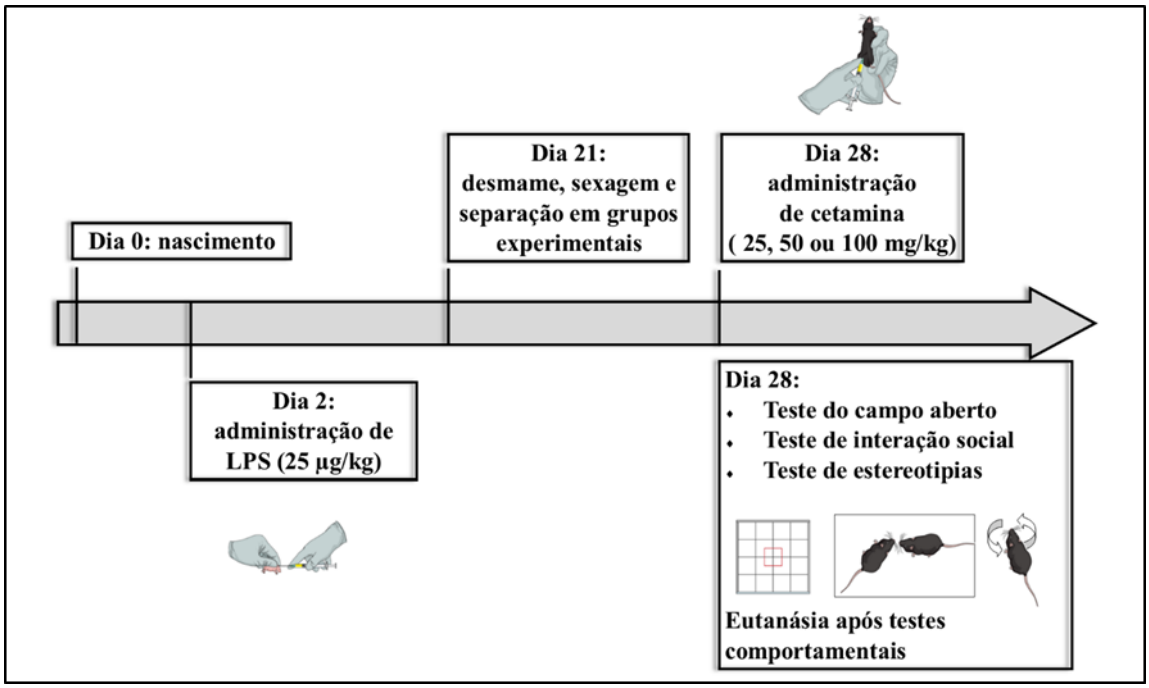
Ao completarem dois dias de vida, a prole (de ambos os sexos) foi retirada da caixa para a exposição à endotoxemia (ou PBS). A endotoxemia foi induzida com uma única administração subcutânea de 25 µg/kg de LPS[51]**.** O LPS foi preparado com o auxílio de PBS para diluição. Os animais controles receberam apenas PBS no mesmo volume administrado no LPS. Logo em seguida, retornaram as suas caixas até completarem 21 dias de vida, quando foram separados por sexo e alocados em caixas próprias, estas contendo 5 animais cada. Neste momento, os animais foram divididos em oito grupos experimentais: PBS + PBS, PBS + Cetamina 25 mg/kg, PBS + Cetamina 50 mg/kg, PBS + Cetamina 100 mg/kg, LPS + PBS, LPS + Cetamina 25 mg/kg, LPS + Cetamina 50 mg/kg, LPS + Cetamina 100 mg/kg.

Ao completarem 28 dias de vida, os animais foram submetidos ao modelo animal de esquizofrenia por Cetamina e receberam três doses em concentrações

diferentes: 25, 50 e 100 mg/kg. O modelo animal de esquizofrenia induzido por cetamina é amplamente validado e replicado, salientando que o modelo adotado para camundongos é divergente do adotado para ratos. Ainda nesse interim, a dose mínima descrita na literatura médica para a indução dos sintomas em camundongos foi de 100 mg/ kg de cetamina[50]**.** Injeções agudas de cetamina (25, 50 ou 100 mg/kg, intraperitoneal (i.p.), CU Chemie Uetikon, Alemanha) foram administradas em um volume de 1 mL/100kg em camundongos adultos jovens. Os testes comportamentais como atividade locomotora, comportamento estereotipado e interação social foram realizados 30 minutos após a injeção de cetamina ou PBS.

Após os testes, os animais foram submetidos à Morte Indolor Assistida (MIA), recebendo injeção de uma dose excessiva de pentobarbital de 80 mg/kg via intraperitoneal de acordo com a resolução 1000, de 12/05/2012 – Conselho Federal de Medicina Veterinária (CFMV), sob a supervisão de médico veterinário responsável.

Figura 1- Desenho experimental



Fonte: autoria própria

3.5 ENSAIOS/TESTES/TÉCNICAS

3.5.1 Testes comportamentais

3.5.1.1 Atividade Locomotora

A tarefa de atividade locomotora avalia o desempenho motor. A habituação a um campo aberto será realizada em um campo aberto de 40 × 40 cm cercado por muros altos feitos de madeira compensada branca com uma parede de vidro frontal. O chão do campo aberto será dividido em 12 retângulos iguais por linhas pretas. Os ratos serão gentilmente colocados no quadrante posterior esquerdo e deverá explorar a arena por 15 min. O número de casos em que os animais cruzarão as linhas pretas serão contados.

3.5.1.2 Comportamento estereotipado

A estereotipia foi definida como um movimento rápido e repetitivo da cabeça e membros anteriores. Os comportamentos pontuados foram cheirar, aparar, balançar a cabeça, mordendo e circulando. Os animais foram colocados em três por gaiola e avaliados quanto ao comportamento estereotipado, conforme descrito por Meller et al. (2004). Os animais foram observados por 1 min em intervalos de 10 min durante 1 h.

3.5.1.3 Interação Social

O teste de interação social constituiu em colocar dois animais de diferentes caixas, mas do mesmo grupo na arena de campo aberto (40 × 40 × 40 cm) por 15 min. Nesse período, foram avaliados três critérios: latência para a primeira interação entre os animais, o número de interações e o tempo total que os animais permaneceram juntos. Antes do teste de interação social, os animais foram socialmente isolados por 6 h.

3.6 VARIÁVEIS DE ESTUDO

Todas as variáveis de estudo referentes a essa pesquisa estão apresentadas no Quadro 2.

Quadro 2 – Variáveis de estudo

Variáveis	**Tipo**	**Natureza**	**Proposta de utilização**
Ativação imune neonatal	Independente	Quantitativa nominal dicotômica	Sim ou Não
Doses de cetamina	Independente	Quantitativa nominal dicotômica	25, 50 ou 100mg/Kg
Atividade locomotora	Dependente	Quantitativa Continua	Número de quadrantes
Interação social	Dependente	Quantitativa Discreta	Número de contatos
Movimentos estereotipados	Dependente	Quantitativa Continua	Quantidade em números absolutos

Fonte: autoria própria

3.7 PROCESSAMENTO E ANÁLISE DOS DADOS

Após a coleta, os dados foram inseridos em um banco de dados, desenvolvido em meio eletrônico, no software IBM SPSS Statistics 24.0 (@copyright IBM corporations and its licensors 1989, 2016). Foi realizado um teste de normalidade de Shapiro-Wilk para caracterização dos dados. Os resultados dos dados paramétricos, foram apresentados como a média ± desvio padrão da média. A análise estatística dos dados entre os grupos foi realizada por meio do teste de ANOVA com post-hoc Tukey. A significância estatística foi considerada para valores de $p<0,05$.

3.8 ASPECTOS ÉTICOS DA PESQUISA

O projeto foi submetido à avaliação da Comissão de Ética no Uso de Animais (CEUA) da UNISUL, seguindo os Princípios de Cuidados de Animais de Laboratório (do inglês Principles of Laboratory Animal Care, Instituto Nacional de Saúde dos EUA, NIH) e aprovado sob o nº. de protocolo 13.029.4.08.IV. A experimentação foi realizada de acordo com os aspectos éticos da Diretriz brasileira para o cuidado e a utilização de animais para fins científicos e didáticos – DBCA (2016), do Conselho Nacional de Controle de Experimentação Animal (CONCEA). Esta diretriz, assim como a legislação brasileira, estabelece a responsabilidade primária e garante a adesão aos princípios de substituição, redução e refinamento.

Em consonância à DBCA, foram adotados os procedimentos que visam evitar, terminar, minimizar ou reduzir a dor, desconforto ou distresse do animal, utilizando assim ações como: i) adoção de tratamento para aliviar a dor, o desconforto ou o distresse; ii) interrupção de um procedimento doloroso; iii) exclusão do animal do estudo; ou iv) morte humanitária do animal realizado por médico veterinário responsável. Os pontos finais devem: limitar sofrimentos que não tenham sido previstos; evitar a antecipação da morte desnecessária de animais cujo bem-estar está menos comprometido do que se crê ou antes que o objetivo científico tenha se completado; informar sobre o índice de severidade do procedimento; avaliar melhoramentos potenciais. Ao reconhecer o ponto final humanitário as seguintes ações devem ser tomadas: deixar de ser o animal um sujeito experimental; ajustar o protocolo para reduzir ou remover a causa do efeito adverso e com isto permitir que o animal se recupere; administrar tratamentos sintomáticos ou de suporte; submeter o animal à morte humanitária. Deve-se destacar que não pode haver demora entre reconhecer e agir. O bem-estar animal não é protegido por sistemas nos quais as decisões e as ações exijam longos comunicados ou burocracia demorada.

4. ARTIGO

LIPOPOLYSACCHARIDE (LPS) INJECTION IN THE NEONATAL PERIOD INCREASES THE RISK OF SCHIZOPHRENIA-LIKE BEHAVIOR IN ADULTHOOD

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LIPOPOLYSACCHARIDE (LPS) INJECTION IN THE NEONATAL PERIOD INCREASES THE RISK OF SCHIZOPHRENIA-LIKE BEHAVIOR IN ADULTHOOD

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ABSTRACT

Despite advances in medical care, neonatal immune activation continues to be a common and significant cause of mortality and morbidity among infants. Activation of the immune system during early life has been associated with an increased risk of schizophrenia in adulthood. The aim of this study was to evaluate schizophrenia-like behaviour in adult mice following neonatal imune activation. Neonatal male and female C57BL/6 mice at postnatal days 2-3 received an injection of 25 µg of lipopolysaccharide (LPS) or PBS as a placebo. Schizophrenia-like behaviour was induced by ketamine (25, 50, or 100 mg/kg) at postnatal day 28. Tests of locomotor activity, stereotyped behaviour, and social interactions were conducted 30 min after injection of ketamine or saline. Young adult mice that received ketamine in a dose of 50 mg/kg showed an increase in locomotor activity; stereotype scores and contact latency were also significantly higher compared with the control group that received the same ketamine dose. We conclude that exposure to imune neonatal activation during the neonatal period can cause alterations in normal brain development and trigger schizophrenia-like behaviour in adulthood.

Keywords: imune neonatal actiation; schizophrenia-like behaviour; psychiatric disorders; LPS.

5. CONSIDERAÇÕES FINAIS

Diante da hipótese exposta, na qual se questionou o envolvimento da ativação imune neonatal no desenvolvimento da esquizofrenia induzido por cetamina, constatou-se que o insulto primário induzido por LPS resultou em um processo de neuroinflamação na prole onde os animais avaliados apresentaram um comportamento do tipo esquizofrênico quando comparados ao grupo controle que foram avaliados através dos testes de atividade locomotora, interação social e movimentos estereotipados. Em conjunto, os resultados deste estudo sugerem que a ativação precoce do sistema imune durante o processo de neurodesenvolvimento, em especial na fase de distinção neuronal, seguido por um segundo agravo na fase adulta, aumenta o risco de desenvolver transtornos psiquiátricos devido a vulnerabilidade do SNC através da neuroinflamação, resultando nos parâmetros relacionados à esquizofrenia após insulto.

5.1 PERSPECTIVAS FUTURAS

Espera-se que este estudo possa servir para futuras pesquisas, com o intuito de compreender os mecanismos envolvidos na relação entre ativação imune neonatal e a esquizofrenia. Além, do fortalecimento da educação para gestantes, bem como ampliação de políticas públicas que possam culminar na diminuição da incidência da doença e ampliação nos estudos epidemiológicos, sendo eles essenciais para a investigação dos agravos em saúde mental, que ainda são escassos.

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APÊNDICE

APÊNDICE A – Normas da revista escolhida para submissão do artigo resultantes da dissertação

Instructions for Authors

Types of papers

Neurochemical Research publishes papers that conform to any of the following categories:

• Original Articles reflect results of original research, and may be of any length but should not usually exceed 8000 words

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Papers should be as brief as is consistent with clear presentation. Preliminary communications are not accepted.

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Manuscript Submission

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Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:

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2. This result was later contradicted by Becker and Seligman [5].

3. This effect has been widely studied [1-3, 7].

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rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

Ideally, the names of all authors should be provided, but the usage of "et al" in long author lists will also be accepted:

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- Book

  South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter

  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

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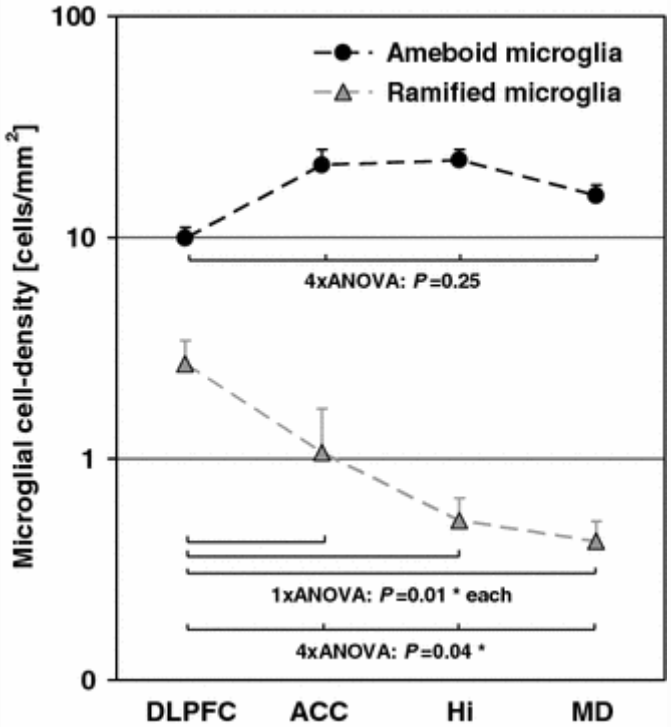

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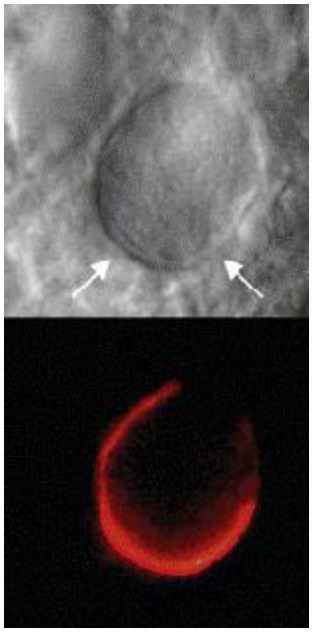

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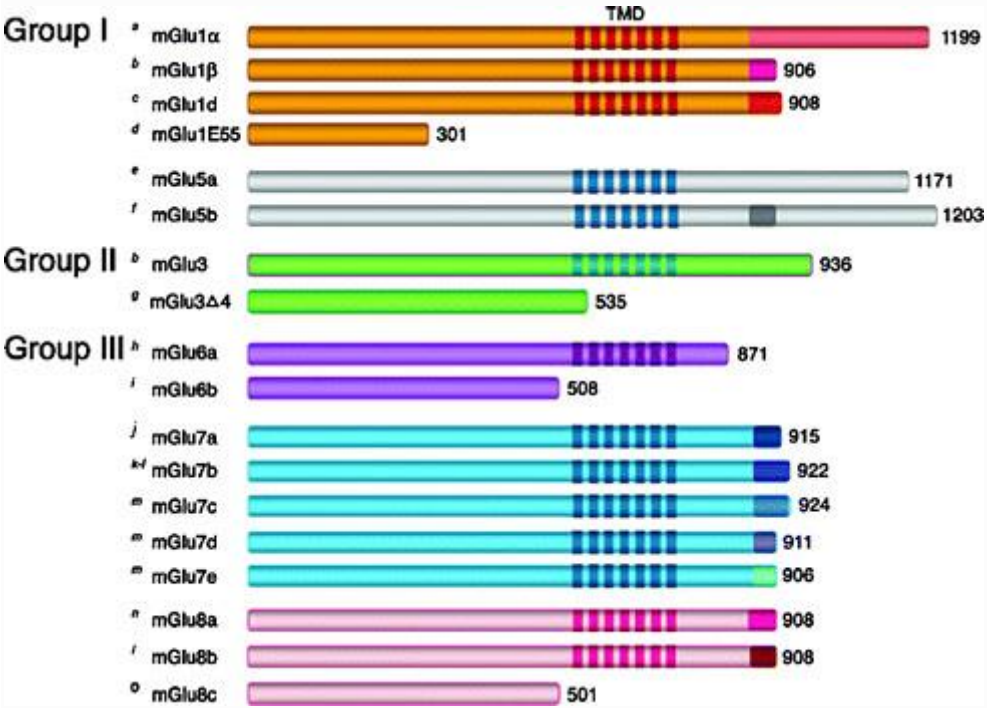

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The trial registration number (TRN) and date of registration should be included as the last line of the manuscript abstract.

For clinical trials that have not been registered prospectively, authors are encouraged to register retrospectively to ensure the complete publication of all results. The trial registration number (TRN), date of registration and the words 'retrospectively registered' should be included as the last line of the manuscript abstract.

Standards of reporting

Springer Nature advocates complete and transparent reporting of biomedical and biological research and research with biological applications. Authors are recommended to adhere to the minimum reporting guidelines hosted by the EQUATOR Network when preparing their manuscript.

Exact requirements may vary depending on the journal; please refer to the journal's Instructions for Authors.

Checklists are available for a number of study designs, including:

Randomised trials (CONSORT) and Study protocols (SPIRIT)

Observational studies (STROBE)

Systematic reviews and meta-analyses (PRISMA) and protocols (Prisma-P)

Diagnostic/prognostic studies (STARD) and (TRIPOD)

Case reports (CARE)

Clinical practice guidelines (AGREE) and (RIGHT)

Qualitative research (SRQR) and (COREQ)

Animal pre-clinical studies (ARRIVE)

Quality improvement studies (SQUIRE)

Economic evaluations (CHEERS)

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Ethics approval'.

Examples of statements to be used when ethics approval has been obtained:

• All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of the Medical University of A (No. ...).

• This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University B (Date.../No. ...).

• Approval was obtained from the ethics committee of University C. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

• The questionnaire and methodology for this study was approved by the Human Research Ethics committee of the University of D (Ethics approval number: ...).

Examples of statements to be used for a retrospective study:

• Ethical approval was waived by the local Ethics Committee of University A in view of the retrospective nature of the study and all the procedures being performed were part of the routine care.

• This research study was conducted retrospectively from data obtained for clinical purposes. We consulted extensively with the IRB of XYZ who determined that our study did not need ethical approval. An IRB official waiver of ethical approval was granted from the IRB of XYZ.

• This retrospective chart review study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Human Investigation Committee (IRB) of University B approved this study.

Examples of statements to be used when no ethical approval is required/exemption granted:

• This is an observational study. The XYZ Research Ethics Committee has confirmed that no ethical approval is required.

• The data reproduced from Article X utilized human tissue that was procured via our Biobank AB, which provides de-identified samples. This study was reviewed and deemed exempt by our XYZ Institutional Review Board. The BioBank protocols are in accordance with the ethical standards of our institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Informed consent

All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. This is especially true concerning images of vulnerable people (e.g. minors, patients, refugees, etc) or the use of images in sensitive contexts. In many instances authors will need to secure written consent before including images.

Identifying details (names, dates of birth, identity numbers, biometrical characteristics (such as facial features, fingerprint, writing style, voice pattern, DNA or other distinguishing characteristic) and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scholarly purposes and the participant (or parent/guardian if the participant is a minor or incapable or legal representative) gave written informed consent for publication. Complete anonymity is difficult to achieve in some cases. Detailed descriptions of individual participants, whether of their whole bodies or of body sections, may lead to disclosure of their identity. Under certain circumstances consent is not required as long as information is anonymized and the submission does not include images that may identify the person.

Informed consent for publication should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort meaning.

Exceptions where it is not necessary to obtain consent:

• Images such as x rays, laparoscopic images, ultrasound images, brain scans, pathology slides unless there is a concern about identifying information in which case, authors should ensure that consent is obtained.

• Reuse of images: If images are being reused from prior publications, the Publisher will assume that the prior publication obtained the relevant information regarding consent. Authors should provide the appropriate attribution for republished images.

Consent and already available data and/or biologic material

Regardless of whether material is collected from living or dead patients, they (family or guardian if the deceased has not made a pre-mortem decision) must have given prior written consent. The aspect of confidentiality as well as any wishes from the deceased should be respected.

Data protection, confidentiality and privacy

When biological material is donated for or data is generated as part of a research project authors should ensure, as part of the informed consent procedure, that the participants are made aware what kind of (personal) data will be processed, how it will be used and for what purpose. In case of data acquired via a biobank/biorepository, it is possible they apply a broad consent which allows research participants to consent to a broad range of uses of their data and samples which is regarded by research ethics committees as specific enough to be considered "informed". However, authors should always check the specific biobank/biorepository policies or any other type of data provider policies (in case of non-bio research) to be sure that this is the case.

Consent to Participate

For all research involving human subjects, freely-given, informed consent to participate in the study must be obtained from participants (or their parent or legal guardian in the case of children under 16) and a statement to this effect should appear in the manuscript. In the case of articles describing human transplantation studies, authors must include a statement declaring that no organs/tissues were obtained from prisoners and must also name the institution(s)/clinic(s)/department(s) via which organs/tissues were obtained. For manuscripts reporting studies involving vulnerable groups where there is the potential for coercion or where consent may not have been fully informed, extra care will be taken by the editor and may be referred to the Springer Nature Research Integrity Group.

Consent to Publish

Individuals may consent to participate in a study, but object to having their data published in a journal article. Authors should make sure to also seek consent from individuals to publish their data prior to submitting their paper to a journal. This is in particular applicable to case studies. A consent to publish form can be found

here. (Download docx, 36 kB)

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Consent to participate' and/or 'Consent to publish'. Other declarations include Funding, Conflicts of interest/competing interests, Ethics approval, Consent, Data and/or Code availability and Authors' contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

Sample statements for **"Consent to participate"**:

Informed consent was obtained from all individual participants included in the study.

Informed consent was obtained from legal guardians.

Written informed consent was obtained from the parents.

Verbal informed consent was obtained prior to the interview.

Sample statements for **"Consent to publish"**:

The authors affirm that human research participants provided informed consent for publication of the images in Figure(s) 1a, 1b and 1c.

The participant has consented to the submission of the case report to the journal.

Patients signed informed consent regarding publishing their data and photographs.

Sample statements if identifying information about participants is available in the article:

Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Images will be removed from publication if authors have not obtained informed consent or the paper may be removed and replaced with a notice explaining the reason for removal.

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A submission to the journal implies that materials described in the manuscript, including all relevant raw data, will be freely available to any researcher wishing to use them for non-commercial purposes, without breaching participant confidentiality.

Data availability

All original research must include a data availability statement. Data availability statements should include information on where data supporting the results reported in the article can be found, if applicable. Statements should include, where applicable, hyperlinks to publicly archived datasets analysed or generated during the study. For the purposes of the data availability statement, "data" is defined as the minimal dataset that would be necessary to interpret, replicate and build upon the findings reported in the article. When it is not possible to share research data publicly, for instance when individual privacy could be compromised, data availability should still be stated in the manuscript along with any conditions for access. Data availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

1. The datasets generated during and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]

2. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

3. All data generated or analysed during this study are included in this published article [and its supplementary information files].

4. The datasets generated during and/or analysed during the current study are not publicly available due [REASON(S) WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.].

5. Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

6. The data that support the findings of this study are available from [THIRD PARTY NAME] but restrictions apply to the availability of these data, which were used under licence for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [THIRD PARTY NAME].

More templates for data availability statements, including examples of openly available and restricted access datasets, are available here:

Data availability statements

Data repositories

This journal strongly encourages that all datasets on which the conclusions of the paper rely are available to readers. We encourage authors to ensure that their datasets are either deposited in publicly available repositories (where available and appropriate) or presented in the main manuscript or additional supporting files whenever possible. Please see Springer Nature's information on recommended repositories.

List of Repositories

General repositories - for all types of research data - such as figshare and Dryad may be used where appropriate.

Data citation

The journal also requires that authors cite any publicly available data on which the conclusions of the paper rely. Data citations should include a persistent identifier (such as a DOI), should be included in the reference list using the minimum information recommended by DataCite, and follow

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Peer reviewers are encouraged to check the manuscript's Data availability statement, where applicable. They should consider if the authors have complied with the journal's policy on the availability of research data, and whether reasonable effort has been made to make the data that support the findings of the study available for replication or reuse by other researchers. Peer reviewers are entitled to request access to underlying data (and code) when needed for them to perform their evaluation of a manuscript.

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ANEXO

ANEXO A - Parecer Aprovação do Comitê de Ética

UNIVERSIDADE DO SUL DE SANTA CATARINA

COMISSÃO DE ÉTICA NO USO DE ANIMAIS – CEUA/UNISUL

Tubarão, 07 de outubro de 2013.

Registro na CEUA (código): 13.029.4.08.IV

Ao pesquisador: Clarissa Martinelli Comim

Curso de Fisioterapia - Campus Universitário Pedra Branca

Prezado(a) Pesquisador(a) ,

Vimos, através deste, informar que o projeto de pesquisa **"A sepse neonatal como fator de risco para o risco desenvolvimento de transtornos psiquiátricos",** foi *aprovado* pela Comissão de Ética no Uso de Animais - CEUA da UNISUL.

A CEUA/UNISUL tem por finalidade cumprir e fazer cumprir, no âmbito da UNISUL e nos limites de suas atribuições, o disposto na legislação federal aplicável à criação e a utilização de animais em atividades de ensino e de pesquisa, realizadas pelos corpos docente, discente e técnico-administrativo da UNISUL e pesquisadores de outras instituições, caracterizando-se a sua atuação como educativa, consultiva, de assessoria e fiscalização nas questões relativas à matéria, sob os aspectos: I - Ético; II - Legal: enquadramento na legislação vigente.

Gostaríamos de salientar que, embora aprovado, qualquer alteração dos procedimentos e metodologias que houver durante a realização do projeto em questão, deverá ser informado imediatamente à Comissão de Ética no Uso de Animais da UNISUL.

Atenciosamente.

Peter Johann Bürger
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