UNIVERSIDADE FEDERAL DA PARAÍBA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA ANIMAL

Efeito do contato humano e da aprendizagem social intra-específica na imobilidade tônica em cobaias (*Cavia porcellus*)

Alan Douglas De Lima Rocha

Areia – PB

2016

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Dissertação apresentada ao Programa de Pós-graduação em Ciência Animal do Centro de Ciências Agrárias da Universidade Federal da Paraíba, como parte das exigências para obtenção do título de Mestre em Ciência Animal.

Areia – PB

2016

Ficha Catalográfica Elaborada na Seção de Processos Técnicos da
Biblioteca Setorial do CCA, UFPB, Campus II, Areia – PB.

R672e Rocha, Alan Douglas de Lima.

Efeito do contato humano e da aprendizagem social intra-específica na imobilidade tônica em cobaias (Cavia porcellus). / Alan Douglas de Lima Rocha. - Areia: UFPB/CCA, 2016.

27 f. : il.

Dissertação (Mestrado em Ciência Animal) - Centro de Ciências Agrárias. Universidade Federal da Paraíba, Areia, 2016.

Bibliografia.

Orientador(a): Luis Felipe Souza da Silva.

1. Cobaias – Imobilidade tônica 2. Porquinho-da-Índia – Contato humano 3. Predador-persa 4. Cavia porcellus – Respostas de IT I. Silva, Luis Felipe Souza da (Orientador) II. Título.

UFPB/CCA CDU: 636.91(043.3)

ALAN DOUGLAS DE LIMA ROCHA

EFEITOS DO CONTATO HUMANO E DA APRENDIZAGEM SOCIAL INTRAESPECÍFICA NA IMOBILIDADE TÔNICA EM COBAIAS (*Cavia porcellus*)

Dissertação apresentada ao Programa de Pós-Graduação em Ciência Animal do Centro de Ciências Agrárias da Universidade Federal da Paraíba, como parte das exigências para a obtenção do título de Mestre em Ciência Animal. Área de Concentração Saúde Animal do brejo paraibano.

APROVADA EM 29/02/2016

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AGRADECIMENTOS

Gostaria de agradecer a todos que fizeram parte da execução deste trabalho. Primeiramente ao Programa de Pós-Graduação em Ciência Animal.

A meu orientador Felipe que sempre me auxiliou não apenas em questões referentes ao trabalho, mas também, em relação à vida.

A professora Leda Menescal, minha coorientadora que me recebeu de braços abertos em seu laboratório na USP de Ribeirão Preto.

Aos técnicos da fisiologia da USP, Mariulza, Rubens Fernando e Leonardo que além de me auxiliar com questões referentes ao trabalho, me ajudaram com informações muito uteis de como viver em Ribeirão e compartilharam um pouco de suas vidas comigo.

À minha família que sempre me apoiou.

A minha namorada Josi, que está sempre comigo, ajudando no que é possível.

Ao Centro de Ciências Agrárias que foi minha casa durante quase 9 anos.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pelo incentivo a pesquisa e pelo auxílio financeiro prestado atreves de bolsa de mestrado.

Aos animais, sem os quais não seria possível a realização deste trabalho e que sempre foram cruciais para o desenvolvimento cientifico.

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RESUMO GERAL

A domesticação de algumas espécies animais, visando o suprimento de demandas humanas se deu há poucos milhares de anos. Contudo, esse tempo não foi suficiente para suprimir diversos mecanismo de defesa desenvolvidos ao longo de milhões de anos. A imobilidade tônica (IT) é uma resposta defensiva inata, caracterizada por um estado de profunda inatividade física e relativa falta de responsividade ao meio ambiente. Estudos revelaram que o contato com seres humanos pode reduzir as respostas de IT em animais. Além disso, a sinalização social do perigo iminente desempenha uma importante função adaptativa anti-predação em muitas espécies. No caso das respostas de medo, os animais podem apresentar comportamento de medo ou não, a partir da observação do comportamento dos membros da mesma espécie. No presente estudo, foi avaliado o efeito do contato humano sobre as respostas de IT em cobaias. Além disso, também estudamos o efeito do convívio entre animais amansados e não amansados nas suas respostas de IT. Para conseguir isso, medimos as respostas de IT induzidas por inversão postural e contenção em cobaias após diferentes tratamentos. No primeiro experimento, foi avaliado o efeito do contato humano sobre as respostas de IT. Para isso, 3 grupos de cobaias foram submetidos a tratamentos diferentes: sem contato, manuseados e amansados. No segundo experimento, foi avaliado o efeito da aprendizagem social na resposta de IT em animais amansados e não amansados que tinham dividido a mesma caixa durante 10 dias. No primeiro experimento, a manipulação ou amansamento por 10 dias não suprimiu a IT, mas aumentou a latência e diminuiu a duração da IT nas cobaias. No segundo experimento, o convívio de animais amansados e não amansados reduziu a duração da IT nas cobaias não amansadas. Estes dados sugerem que ambas as formas de interação humana, pode reduzir o medo do experimentador em cobaias. Parece, portanto, que as cobaias não amansadas aprendem a não temer o experimentador por conviver com cobaias amansadas.

Palavras-chave: Convívio, medo, resposta motora, imobilidade tônica, *Cavia porcellus*, predador-presa

Effects of human contact and intra-specific social learning on tonic immobility in guinea pigs, *Cavia porcellus*

ABSTRACT

The domestication of some animal species, aiming to supply human demands took a few thousand years. This time was not sufficient to remove several defense mechanisms developed over millions of years. Thus, even living in a totally changed environment and often free of any predators, many species still preserve the essential behaviors and former defense mechanisms. These are essentially individual in all species. But many species have social mechanisms aimed at preserving the group. The IT is an innate defense response, characterized by a state of profound physical inactivity and relative unresponsiveness to the environment. This response can be triggered by a wide range of species of vertebrates and invertebrates and is also known as an animal hypnosis or play dead. The IT appears as the last resort used by trapped in extreme effort for survival and aims to reduce the likelihood of attack maintenance, since the movement of the prey stimulates continuity. Studies have shown that contact with humans can reduce IT responses in animals. The social signaling imminent danger plays an important anti-predation adaptive function in many species. In this sense, social learning is a tool used by some species to transmission of environmental information between individuals of the group, quickly and efficiently. In the case of fear responses, animals may exhibit fear or not behavior from observing the behavior of conspecifics. In our study, we evaluated the effect of human contact on the TI responses in guinea pigs. We also evaluated the effect of cohabitation (non-fearful animals with fearful animals) on their TI responses. To achieve this, we measured the TI responses induced by postural inversion and restraint in guinea pigs after different treatments. In our first experiment, we determined the effect of human contact on TI responses by establishing 3 treatment groups: no contact, handled, and tamed. In our second experiment, we addressed the effect of social learning on TI response by testing TI response in tamed and untamed animals that had cohabitated for 10 days. In the first experiment, 10 days of either handling or taming the guinea pigs did not prevent TI, but it did increase latency and decrease duration of the TI behavior in the guinea pigs. In the second experiment, the cohabitation of untamed and tamed animals reduced TI duration in the untamed guinea pigs. These data suggest that both forms of human interaction can reduce experimenter fear in guinea pigs. It therefore seems that untamed guinea pigs learn not to fear the experimenter by cohabitating with tamed guinea pigs.

Keywords: Cohabitation, fear, motor response, tonic immobility, *Cavia porcellus*, predator-prey

CONSIDERAÇÕES GERAIS

Na natureza, em diversos momentos, os animais deparam-se com situações que ameaçam sua integridade física e sobrevivência, como ocorre no confronto com um predador ou um co-específico agressivo. Nessas situações, a execução de respostas comportamentais defensivas adequadas é preponderante para aumentar as chances de sobrevivência do indivíduo durante o confronto. Assim, para lidar com os desafios ambientais, os animais desenvolveram durante a história evolutiva meios anatômicos e comportamentais de defesa (GRAEFF, 1994).

O advento da domesticação de algumas espécies animais, visando o suprimento de demandas humanas se deu há poucos milhares de anos. Tempo ínfimo quando comparado ao período em que estas espécies estiveram livres na natureza. Deste modo, diversos comportamentos e mecanismos de defesa desenvolvidos ao longo de milhões de anos, ainda não foram suprimidos pela seleção promovida pelo homem. Assim, mesmo vivendo em ambiente totalmente modificado e muitas vezes, livre de qualquer predador, muitas espécies ainda preservam os comportamentos e mecanismos de defesa outrora indispensáveis.

Individualmente, as respostas comportamentais defensivas apresentadas podem variar de acordo com alguns fatores, tais como, da distância entre a presa e o predador (RATNER, 1967) e da avaliação do grau de ameaça que cada situação oferece (BLANCHARD; BLANCHARD, 1988). Assim, o primeiro nível de defesa ocorre na vigência de um perigo incerto, seja porque a situação apresenta-se nova, ou porque o predador foi encontrado anteriormente no local. Nessas circunstâncias, a presa executa aproximações cautelosas, com o corpo abaixado e estirado, as orelhas levantadas e farejando continuamente. Esse tipo de resposta foi denominada de avaliação de risco (BLANCHARD; BLANCHARD, 1988). Após a identificação do perigo (presença do predador) e este estando distante, a resposta apresentada pela presa frequentemente é o congelamento. Com a redução da distância entre a presa e o predador, a presa normalmente inicia respostas de fuga e, com uma proximidade maior entre os dois, respostas de luta podem ser executadas. Finalmente, após terem sido capturadas pelo predador algumas espécies emitirão a resposta de imobilidade tônica (IT) (RATNER, 1967; RODGERS; RANDALL, 1987).

A IT é uma resposta defensiva inata, caracterizada por um estado de profunda inatividade física e relativa falta de responsividade ao meio ambiente. Esta resposta pode ser acionada em uma vasta gama de espécies de vertebrados e invertebrados e é também conhecida como hipnose animal ou fingir de morto (RATNER, 1967; THOMPSON et al., 1981). A IT aparece como o último recurso utilizado pela presa num esforço extremo pela sobrevivência e tem por objetivo reduzir a probabilidade de manutenção do ataque, já que a movimentação da presa estimula a sua continuidade. Dessa maneira, o valor adaptativo da IT foi comprovado por diversos estudos (SARGEANT; EBERHARDT, 1975; THOMPSON et al., 1981). Dentre estes estão as observações feitas na natureza por Sargeant e Eberhardt (1975), no qual 58% dos patos que apresentaram IT quando atacados por raposas (*Vulpes fulva*) sobreviveram ao ataque inicial, aumentando, assim, suas chances de sobrevivência. Já pesquisas em laboratório feitas por Thompson e colaboradores (1981) mostraram o valor adaptativo dessa resposta comportamental, onde a permanência das codornas em IT reduziu o tempo em que os gatos permaneciam atacando. Nesse mesmo trabalho, os autores verificaram ainda que os gatos atacavam com maior frequência codornas que estavam em movimento às codornas que haviam emitido a resposta de IT. No laboratório, a IT pode ser induzida por manobras de inversão postural e restrição manual de movimentos. Tais manobras enfatizam as sensações tácteis e proprioceptivas que são importantes para a indução deste comportamento. Estudos recentes correlacionam o comportamento de IT nos animais com sintomas do transtorno de estresse pós-traumático em seres humanos (BOVIN et al., 2014; KALAF et al., 2015; KOZLOWSKA et al., 2015; TEBOCKHORST; O'HALLORAN; NYLINE, 2015).

Quando em grupos, a sinalização social do perigo iminente desempenha uma importante função adaptativa anti-predação em muitas espécies. Neste sentido, a aprendizagem social é uma ferramenta usada por algumas espécies para transmissão de informações sobre o ambiente entre os indivíduos do grupo, de forma rápida e eficiente. Esta permite a rápida transmissão de variantes comportamentais em animais do mesmo grupo, desde peixe até macacos (BAUM, 1969; RENDELL; WHITEHEAD, 2001; WARNER, 1988; WHITEN et al., 1999). Por exemplo, galinhas emitem gritos que evocam respostas de fuga em massa (EVANS; MARLER, 1995); esquilos produzem chamadas de alarme para alertar indivíduos da mesma espécie da presença de predador (SHERMAN PW, 1979); e macacos emitem vocalizações específicas de presença de predador que provocam comportamento defensivo de ameaça em membros do grupo (SEYFARTH; CHENEY, 1992). A sinalização social do perigo (ou medo) também foi pesquisada em ratos de laboratório (*Rattus norvegicus*). John W. Anderson (1954) mostrou que ratos adultos emitiam uma gama de vocalizações ultra-sónicas, sendo a frequência de 22 kHz apontada como um pedido de socorro (ANDERSON, 1954; BORTA; WOHR; SCHWARTING, 2006; EVANS; MARLER,

1995; LITVIN; BLANCHARD; BLANCHARD, 2007). Sabe-se que entre as espécies, quanto maior a aptidão para emitir sinais de perigo social, maior a chance de promover a perpetuação da espécie.

Situações potencialmente danosas evocam uma resposta biológica característica denominada de medo. O medo pode ser adquirido e expresso de diversas formas diferentes (OLSSON; PHELPS, 2007). Pode ser aprendido com a experiência direta (individual) de uma situação adversa. Pode ser socialmente adquirido de instrução por informação verbal (coletivo) ou ainda, a partir da observação do comportamento de um indivíduo da mesma espécie. Desta forma, além dos mecanismos de alerta do grupo, estudos comprovam que o medo pode ser transmitido através da observação de outros que sofrem estímulos aversivos. Assim, Daejong Jeon e colaboradores (2010) mostraram que ratos emitiam comportamento de congelamento ao observarem outros ratos receberem choques nas patas. Além disso, as respostas de medo eram mais intensas quando os observados eram socialmente relacionados, como irmãos ou parceiros de acasalamento. Nesse mesmo estudo, verificou-se ainda que olfato e audição poderiam ter um efeito sinérgico sobre os observadores. Assim, em situações de risco potencial, tanto o alerta de perigo quanto a mensagem de segurança poderiam ser transmitidos através da mudança de comportamento dos outros indivíduos do grupo (BAUM, 1969; COOK; MINEKA, 1990; MINEKA; COOK, 1986, 1993).

Uma questão há muito tempo discutida, é o possível impacto causado pelo contato e manipulação dos animais experimentais pelo experimentador e/ou tratador nas respostas comportamentais estudadas. Estudos mostram que esta manipulação pode causar aumentos significativos em indicadores comportamentais e fisiológicos do stress (GÄRTNER et al., 1980). Além disso, a forma como os animais de laboratório são manuseados pode leva-los a uma relação de medo com os seres humanos (DAVIS; PÉRUSSE, 1988; HURST; WEST, 2010), tornando a manipulação difícil, aumentando o risco de lesões para ambos (experimentador e animal), interferindo, tanto na validade interna quanto externa dos dados da pesquisa (SHERWIN, 2004).

Assim, tendo conhecimento sobre os possíveis efeitos do contato dos humanos com os animais experimentais nas respostas comportamentais de medo, decidimos avaliar o efeito do contato frequente de animais de laboratório com o experimentador e/ou tratador na resposta defensiva de imobilidade tônica em cobaias (*Cavia porcellus*). Além disso, também estudamos o efeito do convívio entre animais amansados e não amansados nas suas respostas de IT.

CAPITULO I

Efeitos do contato humano e da aprendizagem social intra-específica em imobilidade tônica em cobaias (*Cavia porcellus*)

Manuscrito submetido à revista APPLIED ANIMAL BEHAVIOUR SCIENCE

Effects of human contact and intra-specific social learning on tonic immobility in guinea pigs, *Cavia porcellus*

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Abstract

Social learning is the capacity of animals to acquire adaptive information from others. In the case of fear responses, animals can learn fearful or non-fearful responses by observing the behavior of conspecifics. Tonic immobility (TI) is an anti-predatory behavior elicited during intense fear situations. Studies have revealed that contact with humans can reduce TI responses in animals. In our study, we evaluated the effect of human contact on the TI responses in guinea pigs. We also evaluated the effect of cohabitation (non-fearful animals with fearful animals) on their TI responses. To achieve this, we measured the TI responses induced by postural inversion and restraint in guinea pigs after different treatments. In our first experiment, we determined the effect of human contact on TI responses by establishing 3 treatment groups: no contact, handled, and tamed. In our second experiment, we addressed the effect of social learning on TI response by testing TI response in tamed and untamed animals that had cohabitated for 10 days. In the first experiment, 10 days of either handling or taming the guinea pigs did not prevent TI, but it did increase latency and decrease duration of the TI behavior in the guinea pigs. In the second experiment, the cohabitation of untamed and tamed animals reduced TI duration in the untamed guinea pigs. These data suggest that both forms of human interaction can reduce experimenter fear in guinea pigs. It therefore seems that untamed guinea pigs learn not to fear the experimenter by cohabitating with tamed guinea pigs.

Keywords: Cohabitation; Fear; Motor response; Defensive behaviour; Predator-prey

1. Introduction

Social learning is the capacity of animals to rapidly and efficiently acquire adaptive information about their environment from others. Social learning allows intra-population diffusion of behavioral variants in animals, from fish to apes (Baum, 1969; Rendell and Whitehead, 2001; Warner, 1988; Whiten et al., 1999). In the case of fear response, animals can learn fearful or non-fearful behavior by observing how conspecifics respond to a stimulus (Cook and Mineka, 1990; Mineka and Cook, 1993, 1986).

Naturalistic tonic immobility (TI) is an anti-predator behavior characterized by a reversible state of sustained immobility and lack of responsiveness to external stimuli (Klemm, 1971; Ratner, 1967). TI occurs during predator-prey encounters, under conditions of intense fear associated with sensorial stimulation (Bryan Jones and Waddington, 1993;

Gallup, 1979; Kozlowska et al., 2015). It is a terminal defense response with adaptive value to predation as the prey movement stimulates the maintenance of attack by the predator (Gallup, 1979; Thompson et al., 1981). In laboratory experiments, TI can be induced by maneuvers of postural inversion and manual restraint, and has been used to study fear in different species (Donatti and Leite-Panissi, 2011; Narayan et al., 2013; Suzuki et al., 2013; Wang et al., 2014). Recent studies have related peritraumatic TI with post-traumatic stress disorder symptoms in humans (Bovin et al., 2014; Kalaf et al., 2015; Kozlowska et al., 2015).

Although TI is an inborn and phylogenetically old behavior, contact with humans through selection, domestication, manipulation, or socialization can modify its expression (Bryan Jones and Waddington, 1993; Eddy and Gallup, 1994; Suzuki et al., 2013). For example, recent studies have shown that domesticated Bengalese finches have shorter TI episodes compared to its wild ancestor strain (Suzuki et al., 2013). Even in domesticated animals, interaction with humans can affect TI behavior. Passive socialization (Eddy and Gallup, 1994) and regular contact with humans has been shown to reduce TI responses in chicks (Bryan Jones and Waddington, 1992; Jones and Faure, 1981). Although human contact can interfere with the expression of TI behavior, several TI studies have used adult mammals, such as rabbits (Aloisi et al., 1995; Bogdanov et al., 2010; Giannico et al., 2014) or guinea pigs (da Silva et al., 2012; Ferreira and Menescal-de-Oliveira, 2012; Fregoso-Aguilar et al., 2002; Olsen et al., 2002), which have been in regular contact with humans. This suggests that previous human contact during regular maintenance does not interrupt the expression of TI in guinea pigs. Therefore, TI in laboratory animals could be achieved because the novelty of the stimulation necessary to induce TI or the changes in TI due to human contact are specific to the contacting person.

In our first experiment, we evaluated the effect of human contact (no contact, handling, or taming) on the TI responses of guinea pigs. Our hypothesis was that taming would reduce the guinea pigs' fear of humans, resulting in a decrease in TI responses. In our second experiment, we evaluated the effect of cohabitation with non-fearful and fearful animals on the TI responses of guinea pigs.

2. Methods

2.1 Animals

Adult male guinea pigs (*Cavia porcellus*), weighing 400–500 g, were obtained from the Ribeirão Preto School of Medicine's animal care facility. The animals (n = 36) were housed in Plexiglas® cages (56 cm × 37 cm × 39 cm, 5 animals per cage) at 24 ± 1 °C on a 12-h light cycle with free access to water and food. The experimental procedures met the ethical guidelines of the International Society for Applied Ethology. All efforts were made to minimize animal suffering.

2.2 Tonic immobility test

For the TI test, each animal was subjected to 5 episodes of TI, and the latency and duration of the episodes was recorded. We induced TI by holding the animal around the thorax, quickly inverting it, and pressing it down into a V-shaped plywood trough (30 cm × 17 cm × 17 cm). The pressure applied by the hands of the experimenter was proportional to the animal's resistance to the restraining maneuver. The time necessary for the guinea pig to become immobile was recorded as latency. When the animal stopped moving, the experimenter slowly withdrew his hands, and a chronometer was activated to measure the duration (in seconds) of the TI episode, which ended when the animal resumed the upright position. If the animal did not commence TI within 60 s, the duration was assigned a value of zero seconds. The experimenter remained motionless and on sight during TI episodes. For group analysis, the mean of the 5 episodes per animal was considered. It was established that the animals would serve as their own control, which meant that the individuals mean from the baseline test would be compared with its mean from the treatment test. For the TI test, the animals from the same cage were carried to the test room and keep in the test room together in the same transport box.

2.3 Experimental procedures

2.3.1. Experiment 1 – Effect of different levels of human interaction on the latency and duration of TI

For this experiment, 24 animals were divided into 3 treatment groups. For group 1 (no contact group), the animals (n = 8) were submitted to the TI test on days one and eleven, and remained without contact with the experimenter during the interval. For group 2 (handled group), the animals (n = 7) were submitted to the TI test on days one and eleven, and the experimenter was responsible for daily maintenance during the entire experiment. Maintenance involved gently removing the guinea pig from its home box and reallocating it to a clean home box with prefilled food and water tubes. For group 3 (tamed group), the animals (n = 9) were submitted to the TI test on days 1 and 11, and during the interval the animals underwent the taming procedure with the experimenter. For the taming procedure, animals were gently handled and cuddled for 10 minutes, twice a day (08:00 and 14:00), in a quiet and temperature (24 ± 1 °C) controlled room.

2.3.2. Experiment 2 – Effect of taming and cohabitation on the duration of TI

For this experiment, 12 animals were housed in 2 cages (6 per cage). On day one, all animals were submitted to the TI test. For the next 10 days, 3 animals from each cage received the taming procedure (as described in the experiment 1). Following each 10-minute taming session, the animals were returned to their cage to join the other 3 animals that had not received the taming procedure (*i.e.,* untamed). On the eleventh day, the 12 animals underwent the TI test in order to evaluate the effect of taming and cohabitation.

2.4 Statistical analysis

The TI results are reported as means ± standard error of the mean (S.E.M.) for the mean latency and duration of the five TI episodes. The data distribution was analyzed using the Kolmogorov–Smirnov normality test. The differences were analyzed using a paired t-test, with the level of significance set at $p < 0.05$.

3. Results

Experiment 1 – Effect of different levels of human interaction on TI responses

During the first taming session, the guinea pigs were stressed and remained immobile for most of the session, with no exploratory activity. After 3 taming sessions, the animals no longer showed any signs of distress and began exploring and interacting with the experimenter.

For the no contact treatment, no significant difference in latency was found (paired t-test: $t_7 = 0.389$, $p = 0.708$) between the means from the baseline episodes and those from the episodes 10 days later (Figure 1A). TI could still be induced in all animals after 10 days of human contact; however, in both the handled ($t_6 = 2.72$, $p = 0.035$) and tamed ($t_8 = 2.48$, $p < 0.038$) groups, there was a significant increase in the latency of TI (Figures 1B, C).

As for duration, the no contact group did not experience a significant change ($t_7 = 0.5696$, $p = 0,586$) between the baseline test day and 10 days later (Figure 2A). On the other hand, both the handled and tamed groups reduced their TI duration after contact with the experimenter for 10 days. The paired t-test revealed a significant decrease for both the handled ($t_6 = 6.72$, $p = 0.0005$) and tamed groups ($t_8= 7.378$, $p < 0.0001$) (Figures 2B, C).

Experiment 2 – Effect of taming and cohabitation on the duration of TI

For the second experiment, the paired t-tests revealed that the treatment significantly reduced the duration of TI for both the tamed (paired t-test: $t_5= 3.255$, $p < 0.023$) and untamed ($t_4 = 4.689$, $p = 0.009$) animals (Figures 3A, B).

4. Discussion

Our study revealed that regular contact with humans (handling or taming) increased the latency, and reduced the duration, of tonic immobility in guinea pigs. Additionally, our second experiment revealed that the cohabitation of tamed and untamed guinea pigs reduced the duration of TI for the untamed animals without interfering in the reduction experienced by the tamed subjects. To our knowledge, this is the first study that has shown a reduction in TI fear response by cohabitation of fearful animals with non-fearful animals.

In nature, tonic immobility is used as a last resort during a predator-prey interaction and is mediated by an intense fear associated with tactile and proprioceptive stimulation. In our laboratory experiment, the experimenter was the one that induced the TI in the subjects, and therefore we considered the experimenter as a source of fear for the guinea pigs. By using the experimenter as our stimulus, we were able to compare fear responses before and after different manipulations.

In our study, both regular handling and taming were capable of reducing TI behavioral responses, which suggests that both forms of human interaction can reduce fear in guinea pigs. Although the handing and taming treatments were physically different, both forms were considered emotionally positive, and this positive experience likely reduced the level of fear for both groups of guinea pigs. A previous study showed that routine maintenance by caretakers or regular handling reduced TI responses in chicks (*Gallus gallus*) (Bryan Jones and Waddington, 1993; Eddy and Gallup, 1994; Jones and Faure, 1981). In addition, a study on rats found that 17 days of exposure to various types of contact (*e.g.*, motionless hand, restraint, or tickling) reduced the rats' fear of the experimenter (Cloutier et al., 2012).

Our study also showed that although human contact reduced TI responses, it did not prevent the guinea pigs from becoming immobile. This suggests that fear is an important contributor, but not the sole contributor, to TI being induced in guinea pigs. Although TI is a fear-mediated response, it was achieved in our laboratory experiment by postural inversion and restraint, which suggests that tactile and proprioceptive stimuli are also contributors to TI responses. Our study showed that even when fear was reduced by constant human interaction (*i.e.*, tamed guinea pigs), strong sensorial tactile and proprioceptive stimuli could still induce TI responses. Our results could explain why pets, such as rabbits, can be induced into TI by their owners. Additionally, in our study, when the experimenter was different from the caretaker the animals did not change their TI defensive response, suggesting that the reduction in fear was specific to the contacting human. This claim is validated by Gilman et al. (Gilman et al., 1950) who, using habituated adult chickens, found that a change in the experimenter led to the greatest increase in susceptibility to immobility compared to a change in other characteristics of the induction situation.

In our second experiment, we evaluated the effect of social learning on TI response by cohabitating tamed (non-fearful) and untamed (fearful) animals. The cohabitation with tamed animals reduced the TI response in untamed guinea pigs, indicating a possible reduction of fear in untamed animals. This reduction could be related to the cohabitation or, more likely, by the fact that untamed subjects were carried to, and stayed in, the test room with tamed subjects. Supporting our second hypothesis, wild guinea pigs frequently show allelomimetic or contagious behavior, which is displayed by their readiness to follow each other, particularly when frightened (King, 1956). In our case, the tamed animals could have prevented the untamed animals from becoming frightened by the experimenter, thereby reducing their TI responses. In the case of fear responses, studies have suggested that responses can range from fearful to non-fearful depending on the behavior of conspecifics (Baum, 1969; Cook and Mineka, 1990; Mineka and Cook, 1993, 1986). Another hypothesis is that, even without direct contact with the experimenter, untamed animals could have learnt to not fear the experimenter by watching tamed guinea pigs behave calm with the experimenter (observational conditioning). During the 10 days of taming, the untamed animals observed the experimenter removing and replacing the guinea pigs from the home box for the taming procedure. This learned behavior has been shown in rhesus monkeys, which have learnt not to fear snakes by observing non-fearful monkeys (Mineka and Cook, 1986).

In summary, we show that human contact did not prevent TI responses in guinea pigs, but it did increase the latency and decrease the duration, and this is probably due to a reduced fear of the experimenter. Furthermore, cohabitation of tamed and untamed guinea pigs reduced the duration of TI in untamed guinea pigs, which may be contributed to a contagious behavior. Over the last few years, several studies have been conducted to evaluate different aspects of TI fear response, and our study has shown that it is important that interactions with the experimenter and animals from the same cage be taken into consideration. The possibility of a role of social learning in fear response of TI raises a potential field of study about manipulations to reduce fear in captive animals.

Acknowledgements

We would like to thank Mrs. Mariulza Rocha Brentegani for her technical assistance. This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES.

References

Aloisi, A.M., Panerai, A.E., Carli, G., 1995. Effects of an anti-β-endorphin serum on tonic immobility in rabbits. Pharmacol. Biochem. Behav. 51, 577–579. doi:10.1016/0091-3057(94)00370-X

ANDERSON, J.W., 1954. The production of ultrasonic sounds by laboratory rats and other mammals. Science 119, 808–9.

Baum, M., 1969. Extinction of an avoidance response motivated by intense fear: social facilitation of the action of response prevention (flooding) in rats. Behav. Res. Ther. 7, 57–62.

Blanchard, D.C., Blanchard, R.J., 1988. Ethoexperimental approaches to the biology of emotion. Annu. Rev. Psychol. 39, 43–68. doi:10.1146/annurev.ps.39.020188.000355

Bogdanov, A. V, Galashina, A.G., Karamysheva, N.N., 2010. Correlations between neuron activity in the sensorimotor cortex of the right and left hemispheres in rabbits during a defensive dominant and “animal hypnosis”. Neurosci. Behav. Physiol. 40, 801–6. doi:10.1007/s11055-010-9329-x

Borta A, Wohr M, S.R., 2006. Rat ultrasonic vocalization in aversively motivated situations and the role of individual differences in anxietyrelated behavior. Behav Brain Res 166, 271–280.

Bovin, M.J., Dodson, T.S., Smith, B.N., Gregor, K., Marx, B.P., Pineles, S.L., 2014. Does guilt mediate the association between tonic immobility and posttraumatic stress disorder symptoms in female trauma survivors? J. Trauma. Stress 27, 721–4. doi:10.1002/jts.21963

Brudzynski, S.M., Ociepa, D., 1992. Ultrasonic vocalization of laboratory rats in response to handling and touch. Physiol. Behav. 52, 655–60.

Bryan Jones, R., Waddington, D., 1993. Attenuation of the domestic chick’s fear of human beings via regular handling: in search of a sensitive period. Appl. Anim. Behav. Sci. 36, 185–195. doi:10.1016/0168-1591(93)90009-E

Bryan Jones, R., Waddington, D., 1992. Modification of fear in domestic chicks, Gallus gallus domesticus, via regular handling and early environmental enrichment. Anim. Behav. 43, 1021–1033. doi:10.1016/S0003-3472(06)80015-1

Cloutier, S., Panksepp, J., Newberry, R.C., 2012. Playful handling by caretakers reduces fear of humans in the laboratory rat. Appl. Anim. Behav. Sci. 140, 161–171. doi:10.1016/j.applanim.2012.06.001

Cook, M., Mineka, S., 1990. Selective associations in the observational conditioning of fear in rhesus monkeys. J. Exp. Psychol. Anim. Behav. Process. 16, 372–89.

da Silva, L.F.S., Coimbra, N.C., Menescal-de-Oliveira, L., 2012. Rostral ventromedial medulla modulates nociception and tonic immobility behavior through connections with the A7 catecholaminergic region. Behav. Brain Res. 233, 422–7. doi:10.1016/j.bbr.2012.05.020

Davis, H., Pérusse, R., 1988. Human-based social interaction can reward a rat's behavior. Anim. Learn. Behav. 16, 89–92. doi:10.3758/BF03209048

Donatti, A.F., Leite-Panissi, C.R.A., 2011. Activation of corticotropin-releasing factor receptors from the basolateral or central amygdala increases the tonic immobility response in guinea pigs: an innate fear behavior. Behav. Brain Res. 225, 23–30. doi:10.1016/j.bbr.2011.06.027

Eddy, T.J., Gallup, G.G., 1994. Passive socialization to humans: Effects on tonic immobility in chickens (Gallus gallus). Anim. Learn. Behav. 22, 325–331. doi:10.3758/BF03209841

Evans C, M.P., 1995. Language and animal communication: Parallels and contrasts. MIT Press, Massachusetts.

Ferreira, M.D., Menescal-de-Oliveira, L., 2012. Opioidergic, GABAergic and serotonergic neurotransmission in the dorsal raphe nucleus modulates tonic immobility in guinea pigs. Physiol. Behav. 106, 109–16. doi:10.1016/j.physbeh.2012.01.005

Fregoso-Aguilar, T., Urióstegui, T., Zamudio, S., De la Cruz, F., 2002. The differential effect of haloperidol and repetitive induction on four immobility responses in mouse and guinea pig. Behav. Pharmacol. 13, 253–60.

Gallup, G.G., 1979. Tonic immobility as a measure of fear in domestic fowl. Anim. Behav. 27, 316–317. doi:10.1016/0003-3472(79)90159-3

Gärtner, K., Büttner, D., Döhler, K., Friedel, R., Lindena, J., Trautschold, I., 1980. Stress response of rats to handling and experimental procedures. Lab. Anim. 14, 267–74.

Giannico, A.T., Lima, L., Lange, R.R., Froes, T.R., Montiani-Ferreira, F., 2014. Proven cardiac changes during death-feigning (tonic immobility) in rabbits (Oryctolagus cuniculus). J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol. 200, 305–10. doi:10.1007/s00359-014-0884-4

GILMAN, T.T., MARCUSE, F.L., MOORE, A.U., 1950. Animal hypnosis: a study in the induction of tonic immobility in chickens. J. Comp. Physiol. Psychol. 43, 99–111.

Graeff, F.G., 1994. Neuroanatomy and neurotransmitter regulation of defensive behaviors and related emotions in mammals. Brazilian J. Med. Biol. Res. = Rev. Bras. Pesqui. médicas e biológicas / Soc. Bras. Biofísica ... [et al.] 27, 811–29.

Hurst, J.L., West, R.S., 2010. Taming anxiety in laboratory mice. Nat. Methods 7, 825–6. doi:10.1038/nmeth.1500

Jones, R.B., Faure, J.M., 1981. The effects of regular handling on fear responses in the domestic chick. Behav. Processes 6, 135–43. doi:10.1016/0376-6357(81)90032-2

Kalaf, J., Vilete, L.M.P., Volchan, E., Fiszman, A., Coutinho, E.S.F., Andreoli, S.B., Quintana, M.I., de Jesus Mari, J., Figueira, I., 2015. Peritraumatic tonic immobility in a large representative sample of the general population: association with posttraumatic stress disorder and female gender. Compr. Psychiatry 60, 68–72. doi:10.1016/j.comppsych.2015.04.001

King, J.A.., 1956. Social Relations of the Domestic Guinea Pig Living under Semi-Natural Conditions. Ecology 37, 221. doi:10.2307/1933134

Klemm, W.R., 1971. Neurophysiologic studies of the immobility reflex (“animal hypnosis”). Neurosci. Res. (N. Y). 4, 165–212. doi:10.1016/B978-0-12-512504-8.50011-1

Kozlowska, K., Walker, P., McLean, L., Carrive, P., 2015. Fear and the Defense Cascade: Clinical Implications and Management. Harv. Rev. Psychiatry 23, 263–87.

doi:10.1097/HRP.0000000000000065

Litvin, Y., Blanchard, D.C., Blanchard, R.J., 2007. Rat 22kHz ultrasonic vocalizations as alarm cries. Behav. Brain Res. 182, 166–72. doi:10.1016/j.bbr.2006.11.038

Mineka, S., Cook, M., 1993. Mechanisms involved in the observational conditioning of fear. J. Exp. Psychol. Gen. 122, 23–38.

Mineka, S., Cook, M., 1986. Immunization against the observational conditioning of snake fear in rhesus monkeys. J. Abnorm. Psychol. 95, 307–18.

Narayan, E.J., Cockrem, J.F., Hero, J.-M., 2013. Sight of a predator induces a corticosterone stress response and generates fear in an amphibian. PLoS One 8, e73564. doi:10.1371/journal.pone.0073564

Olsen, C.K., Hogg, S., Lapiz, M.D.S., 2002. Tonic immobility in guinea pigs: a behavioural response for detecting an anxiolytic-like effect? Behav. Pharmacol. 13, 261–9.

Olsson, A., Phelps, E. a, 2007. Social learning of fear. Nat. Neurosci. 10, 1095–102. doi:10.1038/nn1968

Ratner, S.C., 1967. Comparative aspects of hypnosis, in: Gordon, J.E. (Ed.), Handbook of Clinical and Experimental Hypnosis. Collier Macmillan Ltd, New York, pp. 550–587.

Rendell, L., Whitehead, H., 2001. Culture in whales and dolphins. Behav. Brain Sci. 24, 309–24; discussion 324–82.

Rodgers RJ, R.J., 1987. Defensive analgesia in rats and mice. Psychol Rec 37, 335–347.

Sargeant AB, E.L., 1975. Death feigning by ducks in response to predation by red foxes (Vulpes fulva). Am Midl. Nat 94, 108–119.

Seyfarth, R.M., Cheney, D.L., 1992. Meaning and mind in monkeys. Sci. Am. 267, 122–8.

Sherman PW, N.M., 1979. Four months of the ground squirrel. Nat. Hist. 88, 50–57.

Sherwin, C., 2004. The influences of standard laboratory cages on rodents and the validity of research data. Anim. Welf. 13, 9–15.

Suzuki, K., Ikebuchi, M., Okanoya, K., 2013. The impact of domestication on fearfulness: a comparison of tonic immobility reactions in wild and domesticated finches. Behav. Processes 100, 58–63. doi:10.1016/j.beproc.2013.08.004

TeBockhorst, S.F., O'Halloran, M.S., Nyline, B.N., 2015. Tonic immobility among survivors of sexual assault. Psychol. Trauma 7, 171–8. doi:10.1037/a0037953

Thompson, R.K.R., Foltin, R.W., Boylan, R.J., Sweet, A., Graves, C.A., Lowitz, C.E., 1981. Tonic immobility in Japanese quail can reduce the probability of sustained attack by cats. Anim. Learn. Behav. 9, 145–149. doi:10.3758/BF03212037

Wang, S., Ni, Y., Guo, F., Sun, Z., Ahmed, A., Zhao, R., 2014. Differential expression of hypothalamic fear- and stress-related genes in broiler chickens showing short or long tonic immobility. Domest. Anim. Endocrinol. 47, 65–72. doi:10.1016/j.domaniend.2013.11.005

Warner, R.R., 1988. Traditionality of mating-site preferences in a coral reef fish. Nature 335, 719–721. doi:10.1038/335719a0

Whiten, A., Goodall, J., McGrew, W.C., Nishida, T., Reynolds, V., Sugiyama, Y., Tutin, C.E., Wrangham, R.W., Boesch, C., 1999. Cultures in chimpanzees. Nature 399, 682–5. doi:10.1038/21415

Figure Captions

Figure 1: Effect of human contact on the latency of TI in guinea pigs. (A) Baseline and post-treatment means ± S.E.M. for guinea pigs that had no contact with the experimenter. (B) Baseline and post-treatment means ± S.E.M. for guinea pigs that were regularly handled by the experimenter. (C) Baseline and post-treatment means ± S.E.M. for guinea pigs tamed by the experimenter. * $p < 0.05$ compared to the baseline.

Figure 2: Effect of human contact on the duration of TI in guinea pigs. (A) Baseline and post-treatment means ± S.E.M. for guinea pigs that had no contact with the experimenter. (B) Baseline and post-treatment means ± S.E.M. for guinea pigs that were regularly handled by the experimenter. (C) Baseline and post-treatment means ± S.E.M. for guinea pigs tamed by the experimenter. * $p < 0.05$ compared to the baseline.

Figure 3: Effect of cohabitation on the duration of TI in tamed and untamed guinea pigs. (A) Means ± S.E.M. for tamed guinea pigs that cohabitated with untamed guinea pigs. (B) Means ± S.E.M. for untamed guinea pigs that cohabitated with tamed guinea pigs. * $p < 0.05$ compared to the baseline.

Figure Captions

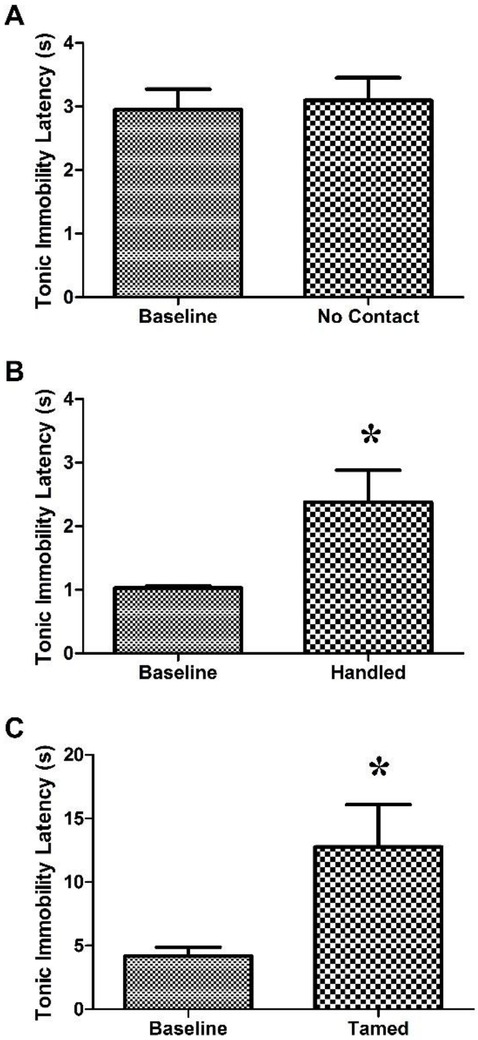

Figure 1: Effect of human contact on the latency of TI in guinea pigs. (A) Baseline and post-treatment means ± S.E.M. for guinea pigs that had no contact with the experimenter (n = 8). (B) Baseline and post-treatment means ± S.E.M. for guinea pigs that were regularly handled by the experimenter (n = 7). (C) Baseline and post-treatment means ± S.E.M. for guinea pigs tamed by the experimenter (n = 9). * $p < 0.05$ compared to the baseline.

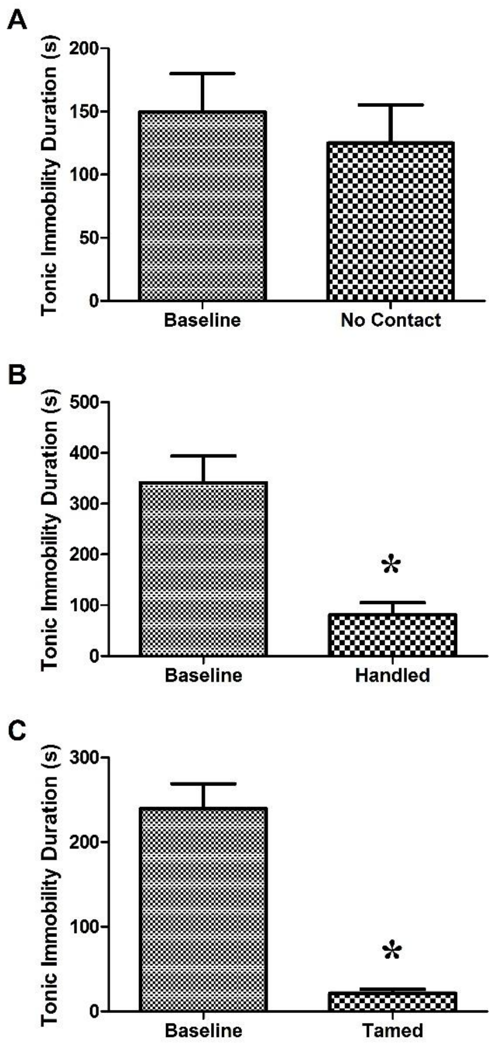

Figure 2: Effect of human contact on the duration of TI in guinea pigs. (A) Baseline and post-treatment means ± S.E.M. for guinea pigs that had no contact with the experimenter (n = 8). (B) Baseline and post-treatment means ± S.E.M. for guinea pigs that were regularly handled by the experimenter (n = 7). (C) Baseline and post-treatment means ± S.E.M. for guinea pigs tamed by the experimenter (n = 9). * $p < 0.05$ compared to the baseline.

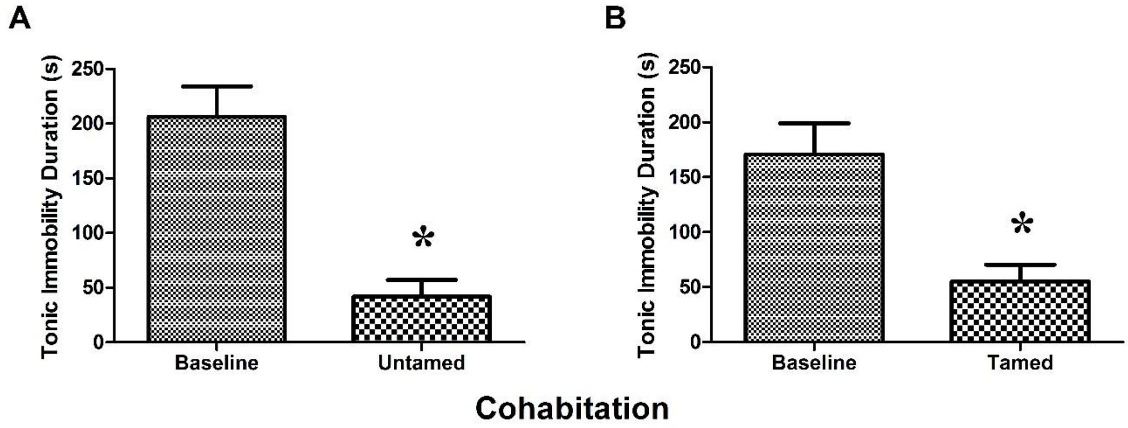

Figure 3: Effect of cohabitation on the duration of TI in tamed and untamed guinea pigs. (A) Means ± S.E.M. for tamed guinea pigs that cohabitated with untamed guinea pigs. (B) Means ± S.E.M. for untamed guinea pigs that cohabitated with tamed guinea pigs. * $p < 0.05$ compared to the baseline (n = 12).

REFERÊNCIAS

ANDERSON, J. W. The production of ultrasonic sounds by laboratory rats and other mammals. **Science (New York, N.Y.)**, v. 119, n. 3101, p. 808–9, 4 jun. 1954.

BAUM, M. Extinction of an avoidance response motivated by intense fear: social facilitation of the action of response prevention (flooding) in rats. **Behaviour research and therapy**, v. 7, n. 1, p. 57–62, fev. 1969.

BLANCHARD, D. C.; BLANCHARD, R. J. Ethoexperimental approaches to the biology of emotion. **Annual review of psychology**, v. 39, p. 43–68, jan. 1988.

BORTA, A.; WOHR, M.; SCHWARTING, R. Rat ultrasonic vocalization in aversively motivated situations and the role of individual differences in anxiety-related behavior. **Behavioural Brain Research**, v. 166, n. 2, p. 271–280, 30 jan. 2006.

BOVIN, M. J. et al. Does guilt mediate the association between tonic immobility and posttraumatic stress disorder symptoms in female trauma survivors? **Journal of traumatic stress**, v. 27, n. 6, p. 721–4, dez. 2014.

COOK, M.; MINEKA, S. Selective associations in the observational conditioning of fear in rhesus monkeys. **Journal of experimental psychology. Animal behavior processes**, v. 16, n. 4, p. 372–89, out. 1990.

DAVIS, H.; PÉRUSSE, R. Human-based social interaction can reward a rat's behavior. **Animal Learning & Behavior**, v. 16, n. 1, p. 89–92, mar. 1988.

EVANS, C.; MARLER, P. **Language and animal communication: Parallels and contrasts**. Massachusetts: MIT Press, 1995.

GÄRTNER, K. et al. Stress response of rats to handling and experimental procedures. **Laboratory animals**, v. 14, n. 3, p. 267–74, jul. 1980.

GRAEFF, F. G. Neuroanatomy and neurotransmitter regulation of defensive behaviors and related emotions in mammals. **Brazilian journal of medical and biological research = Revista brasileira de pesquisas médicas e biológicas / Sociedade Brasileira de Biofísica ... [et al.]**, v. 27, n. 4, p. 811–29, abr. 1994.

HURST, J. L.; WEST, R. S. Taming anxiety in laboratory mice. **Nature methods**, v. 7, n. 10, p. 825–6, out. 2010.

JEON, D. et al. Observational fear learning involves affective pain system and Cav1.2 Ca2+ channels in ACC. **Nature neuroscience**, v. 13, n. 4, p. 482–8, abr. 2010.

KALAF, J. et al. Peritraumatic tonic immobility in a large representative sample of the general population: association with posttraumatic stress disorder and female gender. **Comprehensive psychiatry**, v. 60, p. 68–72, jul. 2015.

KNUTSON, B.; BURGDORF, J.; PANKSEPP, J. Ultrasonic vocalizations as indices of affective states in rats. **Psychological bulletin**, v. 128, n. 6, p. 961–77, nov. 2002.

KOZLOWSKA, K. et al. Fear and the Defense Cascade: Clinical Implications and Management. **Harvard review of psychiatry**, v. 23, n. 4, p. 263–87, 2015.

LITVIN, Y.; BLANCHARD, D. C.; BLANCHARD, R. J. Rat 22kHz ultrasonic vocalizations as alarm cries. **Behavioural brain research**, v. 182, n. 2, p. 166–72, 4 set. 2007.

MINEKA, S.; COOK, M. Immunization against the observational conditioning of snake fear in rhesus monkeys. **Journal of abnormal psychology**, v. 95, n. 4, p. 307–18, nov. 1986.

MINEKA, S.; COOK, M. Mechanisms involved in the observational conditioning of fear. **Journal of experimental psychology. General**, v. 122, n. 1, p. 23–38, mar. 1993.

OLSEN, C. K.; HOGG, S.; LAPIZ, M. D. S. Tonic immobility in guinea pigs: a behavioural response for detecting an anxiolytic-like effect? **Behavioural pharmacology**, v. 13, n. 4, p. 261–9, jul. 2002.

PW, S.; ML, N. Four months of the ground squirrel. **Natural History**, v. 88, p. 50–57, 1979.

RATNER, S. C. Comparative aspects of hypnosis. In: GORDON, J. E. (Ed.). . **Handbook of clinical and experimental hypnosis**. New York: Collier Macmillan Ltd, 1967. p. 550–587.

RENDELL, L.; WHITEHEAD, H. Culture in whales and dolphins. **The Behavioral and brain sciences**, v. 24, n. 2, p. 309–24; discussion 324–82, abr. 2001.

RODGERS, R.; RANDALL, J. Defensive analgesia in rats and mice. **Psychol Rec**, v. 37, p. 335–347, 1987.

SARGEANT, A. B.; EBERHARDT, L. E. Death Feigning by Ducks in Response to Predation by Red Foxes (Vulpes fulva). **American Midland Naturalist**, v. 94, n. 1, p. 108, jul. 1975.

SEYFARTH, R. M.; CHENEY, D. L. Meaning and mind in monkeys. **Scientific American**, v. 267, n. 6, p. 122–8, dez. 1992.

SHERWIN, C. The influences of standard laboratory cages on rodents and the validity of research data. **Animal Welfare**, v. 13, p. 9–15, 2004.

TEBOCKHORST, S. F.; O'HALLORAN, M. S.; NYLINE, B. N. Tonic immobility among survivors of sexual assault. **Psychological trauma : theory, research, practice and policy**, v. 7, n. 2, p. 171–8, mar. 2015.

THOMPSON, R. K. R. et al. Tonic immobility in Japanese quail can reduce the probability of sustained attack by cats. **Animal Learning & Behavior**, v. 9, n. 1, p. 145–149, mar. 1981.

WARNER, R. R. Traditionality of mating-site preferences in a coral reef fish. **Nature**, v. 335, n. 6192, p. 719–721, 20 out. 1988.

WHITEN, A. et al. Cultures in chimpanzees. **Nature**, v. 399, n. 6737, p. 682–5, 17 jun. 1999.