Neuropeptidergic Control of an Internal Brain State Produced by Prolonged Social Isolation Stress

Moriel Zelikowsky,[1] Keke Ding,[1] and David J. Anderson[1,2,3]
[1]*Department of Biology and Biological Engineering, California Institute of Technology, Pasadena, California 91125, USA*
[2]*Howard Hughes Medical Institute, California Institute of Technology, Pasadena, California 91125, USA*
[3]*TianQiao and Chrissy Chen Institute for Neuroscience, California Institute of Technology, Pasadena, California 91125, USA*
Correspondence: wuwei@caltech.edu

Prolonged periods of social isolation can generate an internal state that exerts profound effects on the brain and behavior. However, the neurobiological underpinnings of protracted social isolation have been relatively understudied. Here, we review recent literature implicating peptide neuromodulators in the establishment and maintenance of such internal states. More specifically, we describe an evolutionarily conserved role for the neuropeptide tachykinin in the control of social isolation–induced aggression and review recent data that elucidate the manner by which Tac2 controls the widespread effects of social isolation on behavior in mice. Last, we discuss potential roles for additional neuromodulators in controlling social isolation and a more general role for Tac2 in the response to other forms of stress.

Prior experience, current context, and internal state interact to influence and control behavioral decisions (Anderson and Adolphs 2014). One powerful internal state affecting behavior is that produced by social isolation. Prolonged periods of social isolation exert profound effects on the brain and behavior (House et al. 1988; Hilakivi et al. 1989; Weiss et al. 2004). Despite the abundance of literature establishing the detrimental effects of social isolation on mental health—including an increase in violence, depression, and mortality (House et al. 1988)—relatively little is known about the neurobiology and neurochemistry underlying chronic social isolation stress. Recent studies aimed at understanding the neurobiology underlying social isolation have focused on short periods of isolation (e.g., 24 h) (Matthews et al. 2016), rather than prolonged periods devoid of social contact.

Neuropeptides and other neuromodulators are ideal candidates to mediate internal states (Nitabach and Taghert 2008; Bargmann 2012; Shohat-Ophir et al. 2012; Taghert and Nitabach 2012; Shao et al. 2017). However, whether there are neuropeptides that are specifically involved in mediating effects of prolonged social isolation stress is not clear. Here we discuss the role of neuropeptides as mediators of internal states, highlighting recent studies from our laboratory uncovering a role for the neuropeptide tachykinin-2 in mediating social isolation and its effects on behavior in both mice (Zelikowsky et al. 2018) and fruit flies (Asahina et al. 2014).

NEUROPEPTIDES AS CANDIDATE MEDIATORS OF INTERNAL STATE

Neuromodulators such as biogenic amines and neuropeptides have long been implicated as mediators of internal states (Harris-Warrick and Marder 1991; Bargmann 2012; Marder 2012; Bargmann and Marder 2013; Kennedy et al. 2014). These small molecules have the potential to exert their modulatory effect on brain circuits by activating G protein–coupled receptors, which in turn allow for changes in neuronal excitability and dynamics, thereby altering neural circuit function (Bargmann 2012).

Once a neuropeptide is released it is capable of diffusing across a relatively long range (i.e., μm) to exert its effect, in contrast to fast-acting classical transmitter release (e.g., glutamate, GABA, glycine), which exert their effects at receptors only a short distance from the site of vesicular release (hundreds of nanometers) (Fig. 1; van den Pol 2012). Given that the behavior and function of a hardwired circuit can be altered via neuromodulatory control (Marder 2012), and that neuropeptides are able to exert their effects in a diffuse and slow-acting manner, neuropeptidergic signaling provides an attractive mechanism by which internal state conditions can flexibly and dynamically affect behavior (Hökfelt et al. 2018).

Evidence that neuromodulators regulate internal states and behavior has been provided for a variety of species, behaviors, and neurochemicals (Insel and Young 2000; Bargmann 2012; Marder 2012; Taghert and Nitabach

Published by Cold Spring Harbor Laboratory Press; doi:10.1101/sqb.2018.83.038109

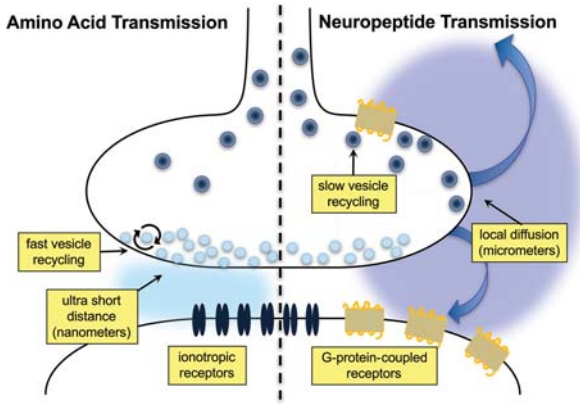

Figure 1. Amino acid (*left*) compared to neuropeptide (*right*) transmission. The postsynaptic effects of amino acid transmission (e.g., glutamate, GABA, glycine) are fast, mediated by ionotropic receptors, and occur across short distances, whereas the postsynaptic effects of neuropeptide transmission are slower, mediated by metabotropic receptors, and exerted at larger distances. (Redrawn from https://quizlet.com/40608575/introduction-to-neuroscience-flash-cards/.)

2012; Kennedy et al. 2014). Indeed, neuropeptides have been implicated in everything from survival-related behaviors such as mating, feeding, and pain to mood, motivation, and reward (van den Pol 2012). Below, we highlight several recent examples.

Flavell et al. (2013) sought to investigate the role of neuromodulation in feeding using *Caenorhabditis elegans* as a model organism. They examined the role of neuromodulators in the control of two foraging states that *C. elegans* switch between—roaming and dwelling. By combining a screen of 57 mutants lacking individual neurotransmitter receptors, neuropeptide receptors, and gap junction subunits with hidden Markov modeling of movement patterns, the authors identified serotonergic signaling and pigment dispersing factor (PDF) signaling as involved in exploratory behavior. Subsequent molecular genetic approaches including optogenetic manipulations revealed parallel and agonistic functions for serotonin and PDF in the control of dwelling and roaming, respectively (Flavell et al. 2013). Given that both dwelling and roaming are enduring behavioral states lasting minutes, the authors argue that the slower time course of neuromodulatory signaling is ideal to convert circuit-based, transiently electrical signals to long-lasting behavior states. These data highlight the role of neuropeptidergic signaling in the control of persistent behavioral states.

Neuropeptidergic signaling has also been shown to control internal states that endure for hours or days. One prime example of this is the discovery that the neuropeptide PDF controls the interaction between pacemaker neurons in the *Drosophila* circadian system (Lin et al. 2004; Nitabach and Taghert 2008; Taghert and Nitabach 2012; Liang et al. 2016). Lin et al. (2004) performed a series of behavioral and immunohistochemical experiments in *Drosophila Pdf* mutants to further examine the neurobiology underlying circadian rhythms. They found that PDF is required to ensure that pacemaker neurons maintain the coordinated, phase-locked activity underlying rhythmic circadian activity. The role of PDF in controlling survival-related behavioral rhythms via its action on pacemaker neurons supports a role for this neuropeptide in modulating long-lasting behavioral states.

TACHYKININ CONTROLS SOCIAL ISOLATION–INDUCED AGGRESSION IN *DROSOPHILA*

One internal state that exerts enduring effects on behavior is that produced by prolonged social isolation. A powerful effect of social isolation on behavior is to promote aggression. This occurs across a variety of species from humans and rodents to *Drosophila* (Arrigo and Bullock 2008; Wang et al. 2008; An et al. 2017). In an effort to identify the neuromodulatory underpinnings of isolation-induced aggression, we focused on the potential role of neuropeptides to mediate this state and performed an unbiased screen of peptidergic neurons and their potential role in promoting aggression in *Drosophila* (Asahina et al. 2014).

The screen revealed that thermogenetic activation of a group of male-specific, fruitless-expressing, *Drosophila* tachykinin (DTK)-containing neurons (Tk^{FruM} neurons) was sufficient to promote aggression in nonaggressive group-housed flies. This effect was further increased when the *DTK* peptide was overexpressed in Tk^{FruM} neurons and combined with thermogenetic activation of these cells (Fig. 2A). Conversely, socially isolated flies bearing

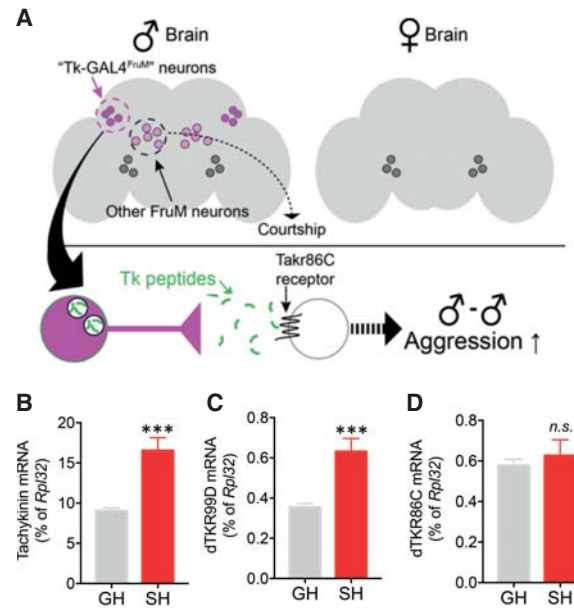

Figure 2. Tachykinin mediates isolation-induced aggression in *Drosophila.* (*A*) Depiction of the role that DTK plays to increase aggression. (*B–D*) Quantification of normalized (*B*) tachykinin, (*C*) dTKR99D, and (*D*) dTKR86C mRNA expression by qRT-PCR, performed on RNA isolated from the heads of group-housed (GH) or single-housed (SH) male flies ($n = 8–9$ trials). Bars represent mean ± SEM. Unpaired *t*-tests. (***) $P < 0.001$; (n.s.) not significant. (*A*, Reprinted from Asahina et al. 2014, with permission from Cell Press.)

overlapping deletions in the *Dtk* gene showed reduced aggression (Asahina et al. 2014). More recently, we have found that the expression of *DTK,* and one of its cognate receptors, *TacR99D*, is up-regulated in socially isolated flies (Fig. 2B,C). Collectively, these data implicate tachykinin as a neuropeptide involved in the control of social isolation–induced aggression in *Drosophila*.

A ROLE FOR TACHYKININ IN MEDIATING SOCIAL ISOLATION–INDUCED AGGRESSION IN MICE

Based on the results in *Drosophila*, we investigated a potential role for tachykinins in controlling isolation-induced aggression in mice (Zelikowsky et al. 2018). In rodents, the tachykinin gene family comprises *Tac1* and *Tac2* (Maggio 1988). As an initial step, mice were either isolated for a period of 2 wk or group-housed, and brains were collected to test for up-regulation of *Tac1* and *Tac2*. We found that *Tac2*, but not *Tac1*, was significantly up-regulated in multiple brain regions following social isolation (Fig. 3; Zelikowsky et al. 2018).

Subsequent loss-of-function experiments showed that perturbations of the Tac2 signaling system, including systemic or intracranial antagonism of Tac2-specific Nk3R receptors via osanetant, chemogenetic silencing of Tac2$^+$ neurons, or Tac2 knockdown using shRNAi, attenuated the effects of social isolation to promote aggression. Conversely, brain-wide chemogenetic activation of Tac2$^+$ neurons combined with overexpression of Tac2 in these same neurons using PHP.B-AAV (a novel viral serotype that crosses the blood–brain barrier [Deverman et al. 2016; Chan et al. 2017]), was sufficient to cause aggressiveness

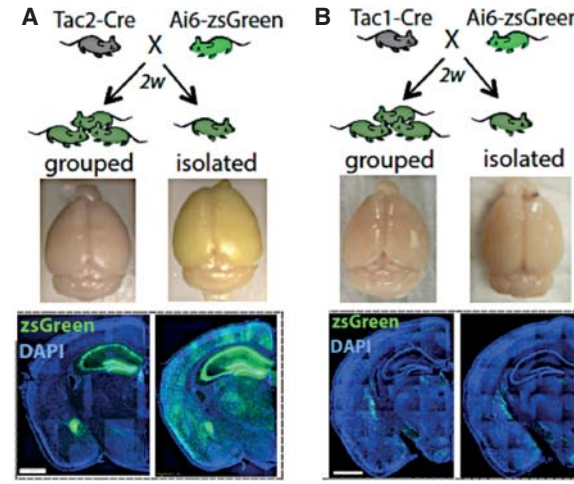

Figure 3. Social isolation up-regulates (*A*) *Tac2*, but not (*B*) *Tac1* in mice. Tac2- or Tac1-Cre mice were crossed to Ai6-zsGreen reporter mice, isolated for 2 wk or group-housed, and zsGreen expression was assessed. (Modified from Zelikowsky et al. 2018, with permission from Cell Press.)

in group-housed mice. This effect was reversed by systemic administration of osanetant (Fig. 4). In contrast, neither activation of Tac2$^+$ neurons nor overexpression of Tac2 on their own was sufficient to produce this effect.

These results are reminiscent of those obtained in flies, in which the mere overexpression of DTK was insufficient to promote aggression, unless combined with the activation of TK^{FruM} neurons. The simplest explanation for this result is that release of the peptide is limiting for its behavioral effects, such that experimentally increasing synthesis

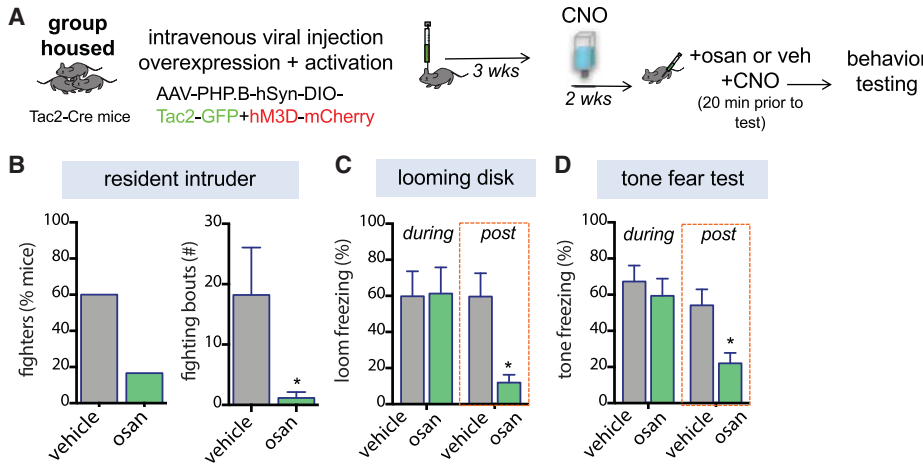

Figure 4. Systemic administration of osanetant reverses the gain-of-function effects of Cre-dependent, brain-wide Tac2 overexpression combined with activation of Tac2$^+$ neurons in group-housed mice. (*A*) Behavioral design. Group-housed Tac2-Cre mice were administered intravenous (retro-orbital) injections of the blood–brain barrier–penetrating viral vectors AAV-PHP.B-hSyn-DIO-Tac2-GFP and AAV-PHP.B-hSyn-DIO-hM3D-mCherry, to overexpress Tac2 and activate Tac2$^+$ neurons, respectively. After 3 wk, animals were put on clozapine N-oxide (CNO) water for 2 wk and injected (i.p.) with an additional dose of CNO before each behavioral test to activate Tac2$^+$ neurons (see Zelikowsky et al. 2018). Experimental mice were treated with an injection of osanetant (i.p.) before testing, to determine whether osanetant could reverse the isolation-like effects produced by Tac2 neuron activation in group-housed mice. Control mice were injected with vehicle. Mice were tested in the resident intruder assay, looming disk assay (Yilmaz and Meister 2013), or tone fear conditioning. Mice treated with osanetant ($n = 6$) showed a reduction in (*B*) enhanced aggression, (*C*) persistent freezing to the looming disk, and (*D*) persistent freezing to the fear conditioned tone, in comparison to vehicle treated mice ($n = 5$). Bars represent mean ± SEM. Unpaired *t*-tests or ANOVA with Bonferroni-corrected post hoc comparisons. (*) $P < 0.05$.

of the peptide has no effect unless there is a concomitant manipulation performed to increase neuronal activity in order to increase the likelihood of peptide release.

Collectively, the results in mice and flies suggest that the tachykinin system mediates at least one of the effects of social isolation (the increase in aggressivity) in multiple species. If the tachykinin system indeed plays a general role in controlling social isolation–induced aggression across species, including humans, it raises the exciting possibility that targeting this system may provide a promising direction for the treatment of mental health disorders related to or caused by social isolation stress (Hökfelt et al. 2003).

Interestingly, previous studies have implicated Tac1/Substance P in rats and cats in the control of aggression (Siegel et al. 1999; Halasz et al. 2009; Katsouni et al. 2009). This suggests either a species difference in the role of Tac1 in aggression (rat and cat vs. mouse) or a potential dissociation between Tac2 and Tac1 in the control of various forms of aggression (e.g., those produced by isolation vs. those produced by other factors, such as sexual experience [Remedios et al. 2017] or territorial competition). Understanding whether the mammalian brain evolved to produce divergent roles for Tac1 and Tac2 in mediating distinct forms of aggression, and if so how, and why, would be an extremely useful step toward understanding particular forms of violence and their underlying neurochemistry.

PROLONGED SOCIAL ISOLATION IN MICE CAUSES A GLOBAL CHANGE IN BRAIN STATE

Social isolation has long been known to promote not only aggression but also a variety of defensive behaviors (Hatch et al. 1963; Valzelli 1969, 1973; Weiss et al. 2004; Matsumoto et al. 2005; Arrigo and Bullock 2008; An et al. 2017). Most investigations have focused on one or two behavioral changes that occur following social isolation. In contrast, we tested a broad array of assays of defensive behaviors and found that prolonged social isolation produced a host of maladaptive effects on such behaviors, including increased foot-shock reactivity, acoustic startle responding, thigmotaxis, and tail rattling, as well as persistent freezing responses to a looming disk, fear conditioned tone, ultrasonic stimulus, or rat presentation (Zelikowsky et al. 2018).

Surprisingly, we found no isolation-evoked changes in anxiety-like behavior using the elevated plus maze assay. This is important because it argues against the idea that the primary effect of social isolation is simply to promote a state of anxiety. In addition, we found that mice spent less time interacting with a novel mouse in a social interaction assay. These later data distinguish our findings from those reported by Matthews et al. (2016), wherein mice isolated for 24 h showed an increase in social interaction when presented with a novel mouse following isolation. These data highlight a potential difference between short periods of social isolation (e.g., 24 h) compared to chronic social isolation (e.g., 2 wk), wherein maladaptive effects on social interactions may begin to emerge.

This widespread effect of social isolation on many facets of behavior suggests that prolonged social isolation generates an internal state that in turn exerts influences over multiple behaviors. Because these behaviors are known to be mediated by different brain regions, it follows that the "state" produced by social isolation must be able to exert its influence via effects on multiple brain regions.

Indeed, when we examined the expression of Tac2 in socially isolated mice using a variety of genetic, molecular, and immunohistochemical approaches, we found that Tac2 was up-regulated across a variety of brain regions involved in emotional processing, including the central amygdala (CeA), dorsal bed nucleus of the stria terminalis, anterior division (dBNSTa), and dorsomedial hypothalamus (DMH) (Zelikowsky et al. 2018). This isolation-induced, widespread up-regulation of Tac2 is consistent with the idea that social isolation generates a global brain state that involves coordinated changes in a variety of brain regions. As described below, our results identify Tac2 as contributing to the neurochemical basis of this internal state, by acting independently in multiple brain regions to influence different isolation-induced behavioral changes. Collectively, these findings tell us that the experience of social isolation changes brain chemistry profoundly, in a way that affects multiple behaviors.

Tac2 ACTS IN A DISTRIBUTED MANNER TO CONTROL THE BRAIN STATE PRODUCED BY ISOLATION

The study of internal states has often focused on one state, one brain region, and one behavior. For example, psychologists often describe a "central motive state," thought to reside in a particular brain region, which coordinates a motivated behavior or set of behaviors. It is tempting to think that such a central state would be implemented via a single, coordinating brain structure, in either a hierarchical or hub-and-spoke-like manner (Fig. 5). However, we found that social isolation stress caused an up-regulation of Tac2 across a number of brain regions, in parallel. These findings suggested that Tac2 could be functioning in a more distributed manner to control the internal state produced by isolation (Fig. 5).

To test this, we performed a series of multiplexed, focal loss-of-function experiments examining the necessity of Tac2 signaling in social isolation stress, and found that Tac2 signaling in different brain regions controlled distinct isolation-induced behaviors. More specifically, in the dBNSTa, CeA, and DMH, Tac2 mediated persistent freezing to innate and conditional fear stimuli, acute freezing to a fear stimulus, and enhanced aggression, respectively (Zelikowsky et al. 2018). Importantly, we found a triple dissociation for the role of Tac2 in each of these regions, suggesting that Tac2 works in a distributed manner to mediate social isolation stress.

This finding of distributed control of brain state by Tac2 contributes to a changing view of the architecture of internal states controlled by peptide neuromodulation. Instead of acting in a unitary, central locus that serves as the

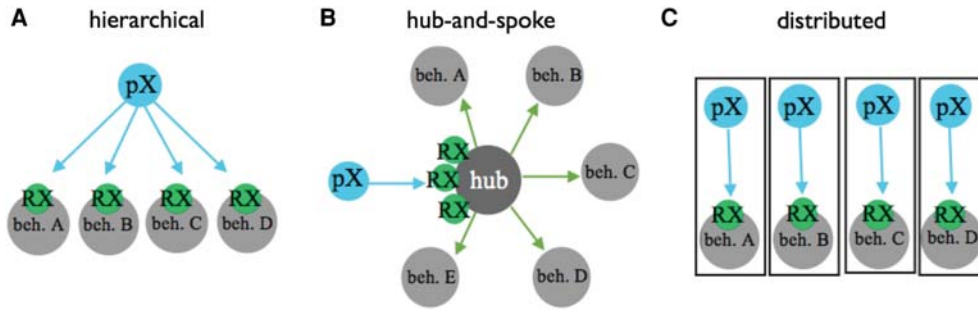

Figure 5. Models for potential neuropeptidergic control of behavior. A neuropeptide may control behavior via a (*A*) hierarchical, (*B*) hub-and-spoke, or (*C*) distributed model. (Adapted from Zelikowsky et al. 2018, with permission from Cell Press.)

hub of the state produced by social isolation, we find that the peptide mediates the effect of the state by acting in a distributed manner, creating a brain-wide neurochemical web that encodes and controls the effects of social isolation stress. Precedent for such a distributed architecture of neuropeptide control has been seen in other systems, such as the influence of PDF on circadian circuits in flies (Lin et al. 2004; Dubowy and Sehgal 2017).

One evolutionary advantage of having an internal state comprised of a neurochemical web across the brain, rather than residing in a central hub, is that it allows for a variety of different, potentially unrelated behaviors to be coordinated but independently controlled. For example, the lack of strong reciprocal connectivity between $Tac2^{+}$ cells in DMH and CeA/dBNSTa (Zelikowsky et al. 2018) implies that Tac2 functions independently in these regions. Therefore, the cooccurrence of persistent fear and enhanced aggression during social isolation reflects a coordinated up-regulation of Tac2 in these distinct brain regions. The mechanisms underlying these coordinated changes in Tac2 expression remain to be elucidated.

CRH, Tac2, AND SOCIAL ISOLATION

Although Tac2 clearly plays an important role in prolonged social isolation stress, our data do not exclude the possibility that additional signaling molecules play a role in controlling this form of stress. One such candidate molecule is corticotropin releasing hormone (CRH). Given CRH's well-known role in mediating stress (Kormos and Gaszner 2013; Witkin et al. 2014; Kash et al. 2015; Chen 2016), it is natural to think that it too might underlie the effects of prolonged isolation stress.

As a first step toward examining the respective roles of CRH and Tac2 in mediating effects of social isolation, we performed *dFISH* analyses and found that ~50% of cells across dBNSTa and CeA coexpress Tac2 and CRH, whereas virtually no $Tac2^{+}$ cells in DMH express CRH. Therefore, in the case of social isolation–induced aggression (which is mediated by DMH), it is unlikely that local up-regulation of CRH contributes to the effects of Tac2. However, with respect to defensive behaviors mediated by dBNSTa and CeA, the data raise the possibility that CRH may act genetically upstream or downstream from Tac2 to control the effects of social isolation stress on behavior. Further epistatic experiments testing whether activation of one system in group-housed mice could be reversed by antagonism of the alternate system will be required to elucidate the relationship between Tac2 and CRH in controlling the behavioral effects of social isolation.

One interesting possibility would be that CRH controls the acute effects of stress, whereas Tac2 controls the more long-term effects of social isolation. This would be consistent with the prevailing view of CRH in controlling acute stress (Chen 2016), and it would also explain why CRH antagonists have failed at relieving the effects of long-term stress in clinical trials (Spierling and Zorrilla 2017).

Tac2 AND OTHER FORMS OF STRESS

In this review we have highlighted the role of Tac2 in mediating social isolation stress. However, these data do not preclude the potential of Tac2 to mediate responses to other stressors. Indeed, a number of pieces of data support this idea. First, we found that the effects of unpredictable footshock to promote persistent freezing to a looming disk was attenuated by administration of osanetant (Zelikowsky et al. 2018), which antagonizes Nk3Rs. Second, data from Ressler and colleagues (Andero et al. 2014, 2016) implicate CeA Tac2 in the influence of immobilization stress on fear memory consolidation. Collectively, these data point to a potential role of Tac2 in mediating the effects of multiple forms of stress.

One interesting possibility is that Tac2 plays a role in mediating prolonged or repetitive forms of stress, rather than acute or singular episodes of stress. Importantly, we found that as the duration of social isolation stress increased, Tac2 expression increased in parallel (Zelikowsky et al. 2018). Similarly Andero et al. (2016) found that Tac2 expression in CeA was enhanced following repeated episodes of stress (immobilization stress followed by fear conditioning) compared with just a single stressful experience. Further experiments contrasting various forms of acute and prolonged stress would be required to test this idea.

CONCLUSION

Neuropeptides provide ideal candidates for integrating environmental, contextual, and experiential factors, medi-

ating internal states, and translating these effects into behavioral output (Hökfelt et al. 2000). Here we review the role of Tac2 in controlling the effects of prolonged social isolation stress on behavior, identifying a similar role for this molecule in *Drosophila* and mice in the control of isolation-induced aggression. We describe the distributed and dissociable manner by which Tac2 mediates the behavioral effects of social isolation in mice, furthering the idea that internal states may be formed by neuropeptidergic "webs" rather than residing in regional "hubs." Importantly, we highlight the notion that Tac2 may be one of a number of neuromodulators controlling social isolation, and that Tac2 may play a more general role in stress. We believe that further investigation of Tac2, as well as of other neuromodulators underlying social isolation stress, will provide critical advances toward understanding the complex state produced by isolation. This in turn may reveal potential approaches toward the treatment of isolation-induced or comorbid mental health disorders.

REFERENCES

An D, Chen W, Yu DQ, Wang SW, Yu WZ, Xu H, Wang DM, Zhao D, Sun YP, Wu JC, et al. 2017. Effects of social isolation, re-socialization and age on cognitive and aggressive behaviors of Kunming mice and BALB/c mice. *Anim Sci J* **88:** 798–806. doi:10.1111/asj.12688

Andero R, Dias BG, Ressler KJ. 2014. A role for *Tac2*, NkB, and Nk3 receptor in normal and dysregulated fear memory consolidation. *Neuron* **83:** 444–454. doi:10.1016/j.neuron.2014.05.028

Andero R, Daniel S, Guo JD, Bruner RC, Seth S, Marvar PJ, Rainnie D, Ressler KJ. 2016. Amygdala-dependent molecular mechanisms of the Tac2 pathway in fear learning. *Neuropsychopharmacology* **41:** 2714–2722. doi:10.1038/npp.2016.77

Anderson DJ, Adolphs R. 2014. A framework for studying emotions across species. *Cell* **157:** 187–200. doi:10.1016/j.cell.2014.03.003

Arrigo BA, Bullock JL. 2008. The psychological effects of solitary confinement on prisoners in supermax units: reviewing what we know and recommending what should change. *Int J Offender Ther Comp Criminol* **52:** 622–640. doi:10.1177/0306624X07309720

Asahina K, Watanabe K, Duistermars BJ, Hoopfer E, González CR, Eyjólfsdóttir EA, Perona P, Anderson DJ. 2014. Tachykinin-expressing neurons control male-specific aggressive arousal in *Drosophila*. *Cell* **156:** 221–235. doi:10.1016/j.cell.2013.11.045

Bargmann CI. 2012. Beyond the connectome: how neuromodulators shape neural circuits. *BioEssays* **34:** 458–465. doi:10.1002/bies.201100185

Bargmann CI, Marder E. 2013. From the connectome to brain function. *Nat Methods* **10:** 483–490. doi:10.1038/nmeth.2451

Chan KY, Jang MJ, Yoo BB, Greenbaum A, Ravi N, Wu WL, Sánchez-Guardado L, Lois C, Mazmanian SK, Deverman BE, et al. 2017. Engineered AAVs for efficient noninvasive gene delivery to the central and peripheral nervous systems. *Nat Neurosci* **20:** 1172–1179. doi:10.1038/nn.4593

Chen A. 2016. Genetic dissection of the neuroendocrine and behavioral responses to stressful challenges. In *Stem cells in neuroendocrinology* (ed. Pfaff D, Christen Y), Springer, Cham.

Deverman BE, Pravdo PL, Simpson BP, Kumar SR, Chan KY, Banerjee A, Wu WL, Yang B, Huber N, Pasca SP, et al. 2016. Cre-dependent selection yields AAV variants for widespread gene transfer to the adult brain. *Nat Biotechnol* **34:** 204–209. doi:10.1038/nbt.3440

Dubowy C, Sehgal A. 2017. Circadian rhythms and sleep in *Drosophila melanogaster*. *Genetics* **205:** 1373–1397. doi:10.1534/genetics.115.185157

Flavell SW, Pokala N, Macosko EZ, Albrecht DR, Larsch J, Bargmann CI. 2013. Serotonin and the neuropeptide PDF initiate and extend opposing behavioral states in *C. elegans*. *Cell* **154:** 1023–1035. doi:10.1016/j.cell.2013.08.001

Halasz J, Zelena D, Toth M, Tulogdi A, Mikics E, Haller J. 2009. Substance P neurotransmission and violent aggression: the role of tachykinin NK_1 receptors in the hypothalamic attack area. *Eur J Pharmacol* **611:** 35–43. doi:10.1016/j.ejphar.2009.03.050

Harris-Warrick RM, Marder E. 1991. Modulation of neural networks for behavior. *Annu Rev Neurosci* **14:** 39–57. doi:10.1146/annurev.ne.14.030191.000351

Hatch A, Wiberg GS, Balazs T, Grice HC. 1963. Long-term isolation stress in rats. *Science* **142:** 507–508. doi:10.1126/science.142.3591.507

Hilakivi LA, Ota M, Lister RG. 1989. Effect of isolation on brain monoamines and the behavior of mice in tests of exploration, locomotion, anxiety and behavioral despair. *Pharmacol Biochem Behav* **33:** 371–374. doi:10.1016/0091-3057(89)90516-9

Hökfelt T, Broberger C, Xu ZQ, Sergeyev V, Ubink R, Diez M. 2000. Neuropeptides–an overview. *Neuropharmacology* **39:** 1337–1356. doi:10.1016/S0028-3908(00)00010-1

Hökfelt T, Bartfai T, Bloom F. 2003. Neuropeptides: opportunities for drug discovery. *Lancet Neurol* **2:** 463–472. doi:10.1016/S1474-4422(03)00482-4

Hökfelt T, Barde S, Xu ZD, Kuteeva E, Rüegg J, Le Maitre E, Risling M, Kehr J, Ihnatko R, Theodorsson E, et al. 2018. Neuropeptide and small transmitter coexistence: fundamental studies and relevance to mental illness. *Front Neural Circuits* **12:** 106. doi:10.3389/fncir.2018.00106

House JS, Landis KR, Umberson D. 1988. Social relationships and health. *Science* **241:** 540–545. doi:10.1126/science.3399889

Insel TR, Young LJ. 2000. Neuropeptides and the evolution of social behavior. *Curr Opin Neurobiol* **10:** 784–789. doi:10.1016/S0959-4388(00)00146-X

Kash TL, Pleil KE, Marcinkiewcz CA, Lowery-Gionta EG, Crowley N, Mazzone C, Sugam J, Hardaway JA, McElligott ZA. 2015. Neuropeptide regulation of signaling and behavior in the BNST. *Mol Cells* **38:** 1–13. doi:10.14348/molcells.2015.2261

Katsouni E, Sakkas P, Zarros A, Skandali N, Liapi C. 2009. The involvement of substance P in the induction of aggressive behavior. *Peptides* **30:** 1586–1591. doi:10.1016/j.peptides.2009.05.001

Kennedy A, Asahina K, Hoopfer E, Inagaki H, Jung Y, Lee H, Remedios R, Anderson DJ. 2014. Internal states and behavioral decision-making: toward an integration of emotion and cognition. *Cold Spring Harb Symp Quant Biol* **79:** 199–210. doi:10.1101/sqb.2014.79.024984

Kormos V, Gaszner B. 2013. Role of neuropeptides in anxiety, stress, and depression: from animals to humans. *Neuropeptides* **47:** 401–419. doi:10.1016/j.npep.2013.10.014

Liang X, Holy TE, Taghert PH. 2016. Synchronous *Drosophila* circadian pacemakers display nonsynchronous Ca^{2+} rhythms in vivo. *Science* **351:** 976–981. doi:10.1126/science.aad3997

Lin Y, Stormo GD, Taghert PH. 2004. The neuropeptide pigment-dispersing factor coordinates pacemaker interactions in the *Drosophila* circadian system. *J Neurosci* **24:** 7951–7957. doi:10.1523/JNEUROSCI.2370-04.2004

Maggio JE. 1988. Tachykinins. *Annu Rev Neurosci* **11:** 13–28. doi:10.1146/annurev.ne.11.030188.000305

Marder E. 2012. Neuromodulation of neuronal circuits: back to the future. *Neuron* **76:** 1–11. doi:10.1016/j.neuron.2012.09.010

Matsumoto K, Pinna G, Puia G, Guidotti A, Costa E. 2005. Social isolation stress-induced aggression in mice: a model to study the pharmacology of neurosteroidogenesis. *Stress* **8:** 85–93. doi:10.1080/10253890500159022

Matthews GA, Nieh EH, Vander Weele CM, Halbert SA, Pradhan RV, Yosafat AS, Glober GF, Izadmehr EM, Thomas RE, Lacy

GD, et al. 2016. Dorsal raphe dopamine neurons represent the experience of social isolation. *Cell* **164:** 617–631. doi:10.1016/j.cell.2015.12.040

Nitabach MN, Taghert PH. 2008. Organization of the *Drosophila* circadian control circuit. *Curr Biol* **18:** R84–R93. doi:10.1016/j.cub.2007.11.061

Remedios R, Kennedy A, Zelikowsky M, Grewe BF, Schnitzer MJ, Anderson DJ. 2017. Social behaviour shapes hypothalamic neural ensemble representations of conspecific sex. *Nature* **550:** 388–392. doi:10.1038/nature23885

Shao L, Saver M, Chung P, Ren Q, Lee T, Kent CF, Heberlein U. 2017. Dissection of the *Drosophila* neuropeptide F circuit using a high-throughput two-choice assay. *Proc Natl Acad Sci* **114:** E8091–E8099. doi:10.1073/pnas.1710552114

Shohat-Ophir G, Kaun KR, Azanchi R, Mohammed H, Heberlein U. 2012. Sexual deprivation increases ethanol intake in *Drosophila*. *Science* **335:** 1351–1355. doi:10.1126/science.1215932

Siegel A, Roeling TA, Gregg TR, Kruk MR. 1999. Neuropharmacology of brain-stimulation-evoked aggression. *Neurosci Biobehav Rev* **23:** 359–389. doi:10.1016/S0149-7634(98)00040-2

Spierling SR, Zorrilla EP. 2017. Don't stress about CRF: assessing the translational failures of CRF_1 antagonists. *Psychopharmacology* **234:** 1467–1481. doi:10.1007/s00213-017-4556-2

Taghert PH, Nitabach MN. 2012. Peptide neuromodulation in invertebrate model systems. *Neuron* **76:** 82–97. doi:10.1016/j.neuron.2012.08.035

Valzelli L. 1969. Aggressive behavior induced by isolation. In *Aggressive behavior* (ed. Garattini S, Sigg EB), pp. 70–76. Excerpta Medica, Amsterdam, The Netherlands.

Valzelli L. 1973. The "isolation syndrome" in mice. *Psychopharmacologia* **31:** 305–320. doi:10.1007/BF00421275

van den Pol AN. 2012. Neuropeptide transmission in brain circuits. *Neuron* **76:** 98–115. doi:10.1016/j.neuron.2012.09.014

Wang L, Dankert H, Perona P, Anderson DJ. 2008. A common genetic target for environmental and heritable influences on aggressiveness in *Drosophila*. *Proc Natl Acad Sci* **105:** 5657–5663. doi:10.1073/pnas.0801327105

Weiss IC, Pryce CR, Jongen-Rêlo AL, Nanz-Bahr NI, Feldon J. 2004. Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. *Behav Brain Res* **152:** 279–295. doi:10.1016/j.bbr.2003.10.015

Witkin JM, Statnick MA, Rorick-Kehn LM, Pintar JE, Ansonoff M, Chen Y, Tucker RC, Ciccocioppo R. 2014. The biology of Nociceptin/Orphanin FQ (N/OFQ) related to obesity, stress, anxiety, mood, and drug dependence. *Pharmacol Ther* **141:** 283–299. doi:10.1016/j.pharmthera.2013.10.011

Yilmaz M, Meister M. 2013. Rapid innate defensive responses of mice to looming visual stimuli. *Curr Biol* **23:** 2011–2015. doi:10.1016/j.cub.2013.08.015

Zelikowsky M, Hui M, Karigo T, Choe A, Yang B, Blanco MR, Beadle K, Gradinaru V, Deverman BE, Anderson DJ. 2018. The neuropeptide Tac2 controls a distributed brain state induced by chronic social isolation stress. *Cell* **173:** 1265–1279 e1219. doi:10.1016/j.cell.2018.03.037